AFOSR-TR- 81-0088

AD A094792

# Response of the Cardiovascular System to Vibration and Combined Stresses

LEVEL II

DDC FILE COPY

PROGRESS REPORT
1979-1980
Air Force Office of Scientific Research

[redacted]

AFOSR-80-0039

Principal Investigator: Charles F. Knapp, Ph.D.

DTIC
ELECTE
FEB 10 1981

Wenner-Gren Research Laboratory
University of Kentucky
Lexington, Kentucky 40506

November, 1980

Approved for public release;
distribution unlimited.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

| REPORT DOCUMENTATION PAGE | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|
| 1. REPORT NUMBER: AFOSR-TR-81-0083 | 2. GOVT ACCESSION NO.: AD-A094 792 | 3. RECIPIENT'S CATALOG NUMBER: |
| 4. TITLE (and Subtitle): Response of the Cardiovascular System to Vibration and Combined Stresses | 5. TYPE OF REPORT & PERIOD COVERED: Interim 10/1/79-9/30/80 |
| | 6. PERFORMING ORG. REPORT NUMBER: |
| 7. AUTHOR(s): C. F. Knapp, J. M. Evans, D. R. Randall | 8. CONTRACT OR GRANT NUMBER(s): AFOSR-80-0039 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS: Wenner-Gren Research Laboratory, University of Kentucky, Rose Street, Lexington, KY 40506 | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 61102F, 2312A2 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS: Air Force Office of Scientific Research (NL), Bolling AFB DC 20332 | 12. REPORT DATE: Nov 80; 13. NUMBER OF PAGES: 58 |
| 14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) | 15. SECURITY CLASS. (of this Report): Unclassified; 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

sinusoidal acceleration, cardiovascular regulation, chronically instrumented, cardiac denervated, total autonomic blockade, left ventricular dimensions, left ventricular volume, arginine vasopressin, plasma volume, renin, epinephrine, norepinephrine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Studies of the previous year indicated an inability of cardiac denervated dogs to maintain stroke volume during the +g$_z$ portion of sinusoidal -2 g$_z$ acceleration stress across the frequency range of 0.004 to 0.25 Hz. To investigate the inadequacy of stroke volume maintenance in these chronically instrumented animals, three cardiac dimensions have been added to provide heart volume information in addition to our standard instrumentation for measuring aortic flow and left and right ventricular and aortic arch pressures.

DD FORM 1 JAN 73 1473



UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

To date, crystals have been implanted to record heart size in 27 animals (16 normal and 11 cardiac denervated) whose body weights ranged from 17 to 26 kg. In the normal dogs, at surgery, major axis averaged 70.1 ± 1.6 mm (S.E.M.), minor axis averaged 53.4 ± 1.1 mm, wall thickness averaged 12.6 ± 0.9 and calculated volumes averaged 25.0 ± 3.5 cc. For cardiac denervated dogs, following denervation surgery, major axis averaged 67.2 ± 1.6 mm, minor axis averaged 55.5 ± 2.3 mm, wall thickness averaged 12.3 ± 0.7 mm and calculated volumes averaged 31.0 ± 6.7 cc. Verifications of actual heart volumes by silastic casts in 10 animals at autopsy indicated a mean volume of 29.1 ± 1.5 cc as compared to average calculated autopsy volumes of 25.6 ± 4.8 cc. During experiments performed on these 10 animals, volumes in the control state averaged 29 ± 5 cc, generally increased with beta blockade (21 ± 7%), decreased slightly with the addition of cholinergic blockade and were slightly enlarged with the addition of α blockade, resulting in the largest control volume seen for the series. In the 11 cardiac denervated dogs, heart size was measured before and immediately after the denervation surgery (a 1 1/2 hour procedure). Heart size was found to generally increase with denervation from 27 ± 7 to 32 to ± 7 cc. Seven normal and 5 cardiac denervated animals have also been studied on the centrifuge. As with our past studies, the protocol consisted of sinusoidal ± 2 $g_z$ acceleration from 0.004 to 0.25 Hz (total time of about 40 min.) followed by a series of 3 min. + and - 2 $g_z$ step inputs in the 1) normal or cardiac denervated animal, 2) beta blocked animal, 3) beta and cholinergically blocked animal and finally, 4) the totally blocked animal (beta, alpha and cholinergically blocked state). An analysis of left ventricular volume changes and corresponding changes in pressure, stroke volume, heart rate and peripheral resistance in response to the acceleration stress and pharmacologic interventions is continuing.

In the second phase of our research effort, acceleration induced changes in circulating levels of arginine vasopressin (ADH), plasma osmolarity, plasma renin activity, plasma volume and circulating epinephrine and norepinephrine have been measured. This investigation was initiated because of an observed, acceleration induced, increase in aortic pressure which was evident within the first few minutes of the onset of centrifugation, persisted throughout the sinusoidal acceleration test period, appeared to be fairly independent of the frequency of acceleration, and remained for several (5 to 10) minutes following the end of the test period. In addition, it appeared to be independent of both cardiac innervation, particularly right heart afferent information and of autonomic effector activity, since it was present to a greater extent in the same dogs following pharmacological blockade of sympathetic α, β and cholinergic activity. The magnitude of the increase in aortic pressure was approximately 15 mm Hg in reflexive dogs, whether normal or cardiac denervated, and more than double that amount in the same dogs in the nonreflexive state.

Analysis of the neurohumoral agents, combined with the dissection of the pressor response into its cardiac output and peripheral resistance components, implicated cardiac output, via neurally-mediated heart rate increases, as responsible for the unblocked pressure increases. In the autonomically blocked animals, the response was due completely to increased peripheral resistance, which correlated with increased plasma levels of arginine vasopressin, but not with increased osmolarity, plasma volume, renin activity, epinephrine or norepinephrine.

## TABLE OF CONTENTS


Form DD 1473 (Abstract) . . . . . i

Introduction . . . . . 1

A. Cardiac Dimension Changes During Dynamic Acceleration Loadings in Normal and Cardiac Denervated Dogs . . . . . 3

Methods . . . . . 4

Surgical Procedures . . . . . 4
Crystal Fabrication and Testing . . . . . 4
Analog Equipment Modifications . . . . . 6
Instrumentation Modifications for Centrifuge Compatibility . . . . . 9
Digital Data Acquisition and Analysis . . . . . 9

Experimental Protocol . . . . . 12

Procedure for Autonomic Blockade . . . . . 12
Test Sequence . . . . . 13

Preliminary Results . . . . . 14

Changes in Left Ventricular Volume (from surgery to autopsy) . . . . . 14
Sinusoidal Acceleration Response . . . . . 20
Response to Rapid Onset Acceleration . . . . . 23

B. Neurohormonal Components of an Acceleration-Induced Pressor Response in Normal and Cardiac Denervated Conscious Dogs . . . . . 29

Methods . . . . . 30

Surgical . . . . . 30

a) Cardiac denervation . . . . . 30
b) Implant procedures . . . . . 31
c) Postoperative care . . . . . 31

Experimental Protocol and Blood Sampling Techniques . . 31

a) Plasma renin activity . . . . . 32
b) Plasma arginine vasopressin activity and osmolality . . . . . 32
c) Plasma norepinephrine and epinephrine activity . . . . . 32
d) Plasma volume and hematocrit . . . . . 32



Accession For
NTIS GRA&I
DTIC TAB
Unannounced
Justification
By
Distribution/
Availability Codes
Dist | Special
A



AIR FORCE OFFICE OF SCIENTIFIC RESEARCH (AFSC)
NOTICE OF TRANSMITTAL TO DDC
This technical report has been reviewed and is approved for public release IAW AFR 190-12 (7b).
Distribution is unlimited.
A. D. BLOSE
Technical Information Officer

Approved for public release;
distribution unlimited.

Total Autonomic Efferent Blockade . . . . . 32

Data Acquisition . . . . . 33

Statistical Analysis . . . . . 34

Results . . . . . 34

Hemodynamic . . . . . 34

Neurohumoral . . . . . 38

a) Plasma volume as indicated by $RISA^{131}$ or hematocrit changes . . . . . 38
b) Plasma renin activity . . . . . 40
c) Plasma arginine vasopressin (ADH) and osmolarity activity . . . . . 40
d) Circulating norepinephrine and epinephrine activity . . . . . 43

Discussion and Conclusions . . . . . 44

References

Appendix

A. Research Team

B. Publications and Presentations

# INTRODUCTION

During the past several years, our experiments have investigated the acceleration induced, pressure, flow and force disturbances which initiate peripheral vascular and cardiac mechanisms by neural, blood borne, and local feedback pathways. With the animal in the normal reflexive state, the frequency response characteristics of the intact cardiovascular system, as determined by heart rate, stroke volume, and total peripheral resistance, have been identified. The frequency response characteristics of the passive circulatory system (following total autonomic blockade) have been established and compared to the animal in the reflexive state to evaluate the effectiveness of neural control mechanisms.

Although quantification of the frequency response of cardiovascular regulation in the reflexive animal is necessary and valuable, it does not however, provide frequency response information concerning specific efferent pathways which contribute to a particular response. To this end, we have developed a cardiac denervated animal preparation in addition to our normal preparation, and have conducted experiments to differentiate between central (i.e., cardiac) and peripheral (i.e., vascular) control mechanisms in the regulation of pressure and cardiac output during $g_z$ acceleration.

Results from these studies have indicated an inability of cardiac denervated dogs to maintain stroke volume during the $+g_z$ portion of sinusoidal $\pm$ 2 $g_z$ acceleration stress across the frequency range of 0.004 to 0.25 Hz. To investigate the inadequacy of stroke volume maintenance in these animals, efforts have begun to provide direct information about left ventricular volume changes in response to acceleration stress. Our chronically-instrumented animal preparation has been enhanced by the addition of three cardiac dimensions to our standard instrumentation for measuring aortic flow and left and right ventricular and aortic arch pressures. From the three dimensions of left

ventricular major and minor axis and wall thickness, volume of the left ventricle and left ventricular inflow are being calculated as a function of time by an on-line analog circuit and an off-line PDP 11/34 computer. (See Section A.)

The second part of our effort deals with the neurohormonal components of the acceleration induced pressor responses that we have observed in both normal and cardiac denervated animals. Specifically, we have consistently observed an increase in mean arterial pressure, from control values, during the time animals were exposed to the oscillatory acceleration loadings. This has been observed for both normal and cardiac denervated animals in both the reflexive and nonreflexive states; suggesting a nonautonomic neural response. As a result, we have investigated changes in circulating levels of arginine vasopressin (ADH), plasma renin activity and/or plasma volume changes and more recently have collaborated with Dr. M. Ziegler (University of Texas) to investigate changes in circulating catecholamines. (See Section B.)

## A. CARDIAC DIMENSION CHANGES DURING DYNAMIC ACCELERATION LOADINGS IN NORMAL AND CARDIAC DENERVATED DOGS

Results from our previous studies have indicated that an intact cardiac regulatory mechanism was essential for maintaining the integrity of the cardiovascular system during low frequency, sinusoidal acceleration stress. Specifically, the inability of cardiac denervated (as opposed to fully innervated) dogs to maintain blood pressure during the +2 $g_z$ portion of the acceleration stress was found to be principally due to the inability of these animals to maintain stroke volume during this time. Mechanisms proposed to explain the inadequacy of stroke volume maintenance included: a smaller end diastolic volume in cardiac denervated dogs implying disconnection of a) an afferent (sensory) mechanism which adjusted venous return or b) an efferent mechanism responsible for adjustment of diastolic (relaxed) heart size, or both. Alternatively, a large end <u>systolic</u> volume at the same or increased end diastolic volume would imply disconnection of an efferent mechanism for adjustment of end systolic volume (the standard sympathetic inotropic pathway). Any or all of these mechanisms could combine to result in a decrease in stroke volume, and for that reason we implemented a procedure to provide direct information about volume changes of the left ventricle in response to acceleration stress. The technique chosen was that of Rankin et al (1) in which pairs of ultrasonic dimension crystals were implanted to record major and minor epicardial dimensions and wall thickness. From these data, the volume was calculated assuming a prolate ellipsoidal geometry. What follows is a description of our progress to date in surgical implant procedures; crystal fabrication and testing; analog equipment modifications to allow for simultaneous measurement of electromagnetic flow, pressure and dimension; modification of instrumentation for centrifuge compatability; digital data acquisition techniques and results from the experiments conducted so far.

## METHODS

### Surgical procedures:

Twenty seven mongrel dogs (17 to 26 kg) have been instrumented for further study. Each animal was anesthetized with sodium thiopental (40 mg), intubated, positive pressure respirated, and an L4 thorocotomy performed under sterile conditions. A pair of 5 mm diameter ultrasonic dimension transducers was positioned to record the maximum anterior to posterior epicardial left ventricular chamber diameter. Another 5 mm pair was positioned to give epicardial major axis diameter (one crystal was placed in the groove between the left atrium and the pulmonary artery and the other was located near the apical dimple). Wall thickness of the left ventricle was measured by a 1 mm diameter crystal tunneled to the endocardial surface of the anterior wall and a 3 mm diameter crystal positioned directly over it. Each pair of crystals was aligned at surgery to give the best received signal possible, and further monitoring was done following placement of the remaining instrumentation (left ventricular pressure gauge, aortic flow probe, right atrial cannula) to ensure continued signal quality. In ten of the animals, a length of tubing was placed in the thoracic cavity to allow for later passage of a micro-manometer to measure thoracic cavity pressure; placement of the other transducers has been detailed in past progress reports. The chest wall was closed, the leads placed in a subcutaneous dacron pouch and the animal was allowed at least three weeks of postoperative recovery. In two animals, the leads ere exteriorized at the time of surgery in order to monitor changes in heart shape and size post-operatively.

### Crystal fabrication and testing:

The transducers used for making dimension measurements are made from type LTZ 2, Lead Titonate Zyrconate (Transducer Products), 5 MHz "thickness

mode" plates of piezoelectric ceramic to which is added a diverging lens on the front surface. At present, the transducers are fabricated by scoring 5 MHz crystal material into 5 mm, 3 mm and 1 mm squares. The corners are then rounded on a lathe or with fingernail clippers and emery board. In order for the wires to be soldered properly, the crystal must be cleaned with acetone and the area to be soldered scraped with a scalpel. The wires to be soldered to the crystal are tinned so that only a quick touch of the soldering iron is required to affix the wire to the material. A plastic resin, which is used to make a lense for the transducer, is mixed with its hardener and placed in a vacuum chamber until completely degassed. A drop of the resin mixture is then placed on the surface of the crystal material to form a lense with a curvature that will transmit a 60° cone of ultrasound. A backing of closed cell foam is epoxyed to the reverse side of the 5 mm and 3 mm transducers to absorb the signal eminating from the back of the crystal material. The transducer is then electrically insulated by applying polystyrene coil dope to exposed areas. To accommodate suturing the transducer to the heart surface, dacron patches are applied to the front and back of the 5 mm and 3 mm crystals with silicone rubber that can be easily sewn through. The front patch has a 5 mm hole for the lens to promote optimal signal transmission.

The 1 mm crystals are slightly different in that thin, stainless steel wire is used and is threaded through a length of tubing to assist in placement through the heart wall. Also, both sides of the crystal material are lensed, because transducer orientation cannot be controlled during surgical placement on the endocardial surface of the left ventricle.

Testing of the finished transducer is accomplished by affixing the transducers to a micrometer placed in a non-reflecting styrofoam tank and recording the signal at known distances apart and at various rotation angles.

To assist in improving transducer design, a laser Schlieren system has been developed and is used to allow for visualization of transducer signal radiation patterns. Through the use of this system, beam angle, intensity and direction of various transducer configurations can be compared and the results recorded on film. By using this technique the output of each transducer can be verified prior to sterilization for surgery.

Analog equipment modifications:

As expected, simultaneous use of the dimension crystals with the electromagnetic flow transducers produce signals which interfered with each other. Conversations with other laboratories and with the manufacturers of our electromagnetic square-wave flow meter (Zepeda Instruments) and our dimension meter (Schussler and Associates) resulted in two suggestions: 1) an outline of possible steps to take to electronically synchronize the two pieces of equipment or 2) manually sample from first one meter and then from the other and assume that the same experimental conditions hold from one time to the next. Due to the nature of our dynamic experimental environment, the second suggestion was rejected and several attempts to synchronize our equipment were made.

The initial suggestions from the manufacturers resulted in a digital circuit which synchronized the electromagnetic drive oscillator and flow gate signal to certain divisions of the dimension clock frequency. This circuit consisted of a divider circuit, a pulse-width and signal shaping circuit and

a low-impedance driving circuit. This device took a signal from the dimension meter at a point in the dimension circuit that had already been divided and after further division by the new circuit, this signal was used to override and capture the electromagnetic oscillator. This synchronizing circuit worked nicely for synchronizing at certain frequencies, but for those frequencies which are necessary for operation of the flowmeter oscillator (a limited operating band) only a portion of the interference could be eliminated. Additionally, the flowmeter gate was not adjustable, further limiting our timing ability.

Given the features of the Zepeda flowmeter in design (square-wave) and accessibility (sealed assemblies) we decided to move to another flowmeter of different design and circuitry. The Biotronix 610 pulsed logic flowmeter which we have used for several years was designed to operate at frequencies near that required for synchronization. Also, schematics were available for its circuit components, and those components were not sealed. After studying the design of this flowmeter, it appeared that its digital circuitry would be more compatible with the digital component circuitry of the dimension meter. Finally, the design of its flow gate was such as to allow for sequential excitation of all four dimension transmitter pulses and the associated crystal ring and therefore make possible the removal of ultrasonic interference from the electromagnetic flow signal (Figure A-1).

With these considerations in mind, a circuit was bread-boarded which used the dimension meter clock pulse (divided down) as the flow oscillator, rather than capturing the flow oscillator as was necessary with the Zepeda meter. This circuit plus an adjustment of the flowmeter resulted in simultaneous dimension and electromagnetic flow readings of excellent quality (see results

FIG. 2: SYN[illegible]RONIZATION

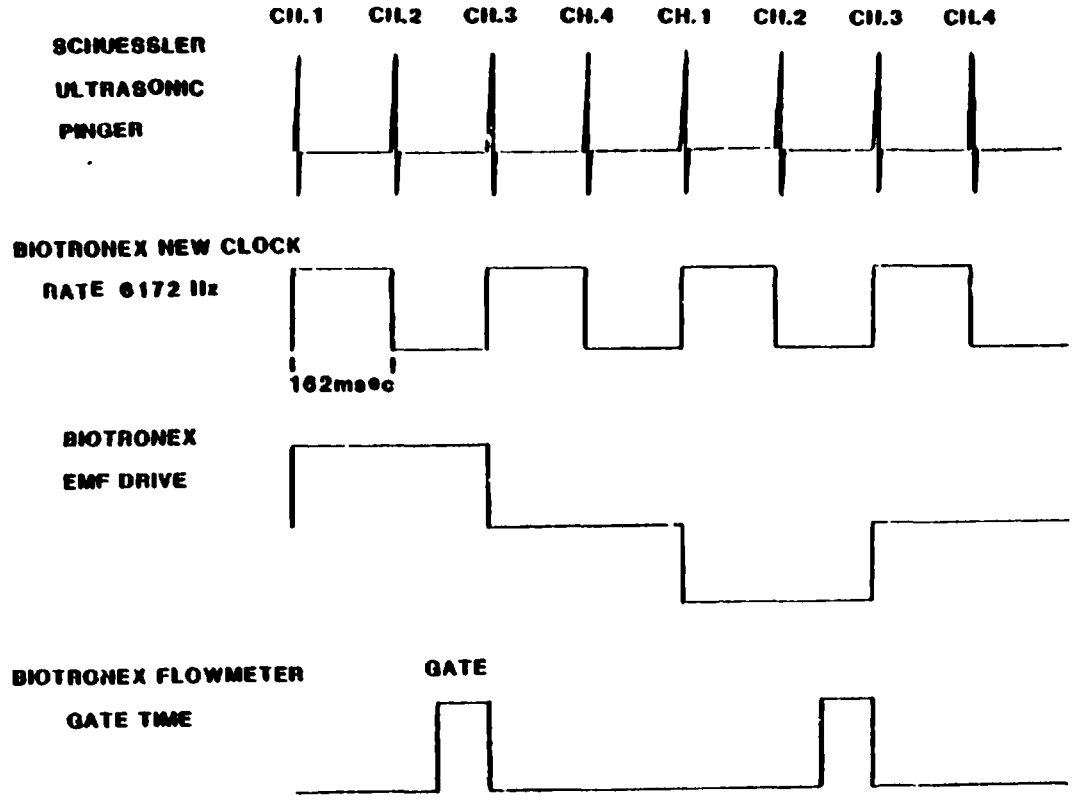


FIGURE A-1

Timing diagram for synchronization of ultrasonic dimension transmitter pulses to electromagnetic flow driver and gate

section). This circuit is basically an analog inverter made from a general purpose operational amplifier with a frequency response high enough to pass an undistorted clock pulse, Figure A-2. These synchronized meters were then modified for mounting on the centrifuge platform and have become a part of our standard centrifuge instrumentation package.

Concern about ultrasonic interference on the pressure signal did not materialize and therefore no corrective action was required.

Instrumentation modifications for centrifuge compatibility:

After verification of signal compability (3 dimensions, electromagnetic flow, 1 Konigsberg and 3 Millar pressures) the dimension and flowmeters were modified to fit the instrumentation bays of the rotating platform on the centrifuge arm. The electromagnetic flowmeter just required a mounting fixture, but the dimension meter required drastic modification. The internal components of the meter and the internal parts were reconfigured in the new space. The smaller box was then fitted with centrifuge mounting brackets that could allow for easy removal of the meter when needed for weekly surgical implantations.

Digital data acquisition and analysis:

Calculations of heart volume were made on an off-line basis using a PDP 11/34 computer. All analog signals including the three heart dimension measurements were recorded on an 14 channel Ampex tape recorder during each experiment. The recorded physiological data were sampled at two millisecond intervals and smoothed with a digital filter. The geometry of the left ventricle was represented as a three-dimensional, prolate ellipsoidal shell.

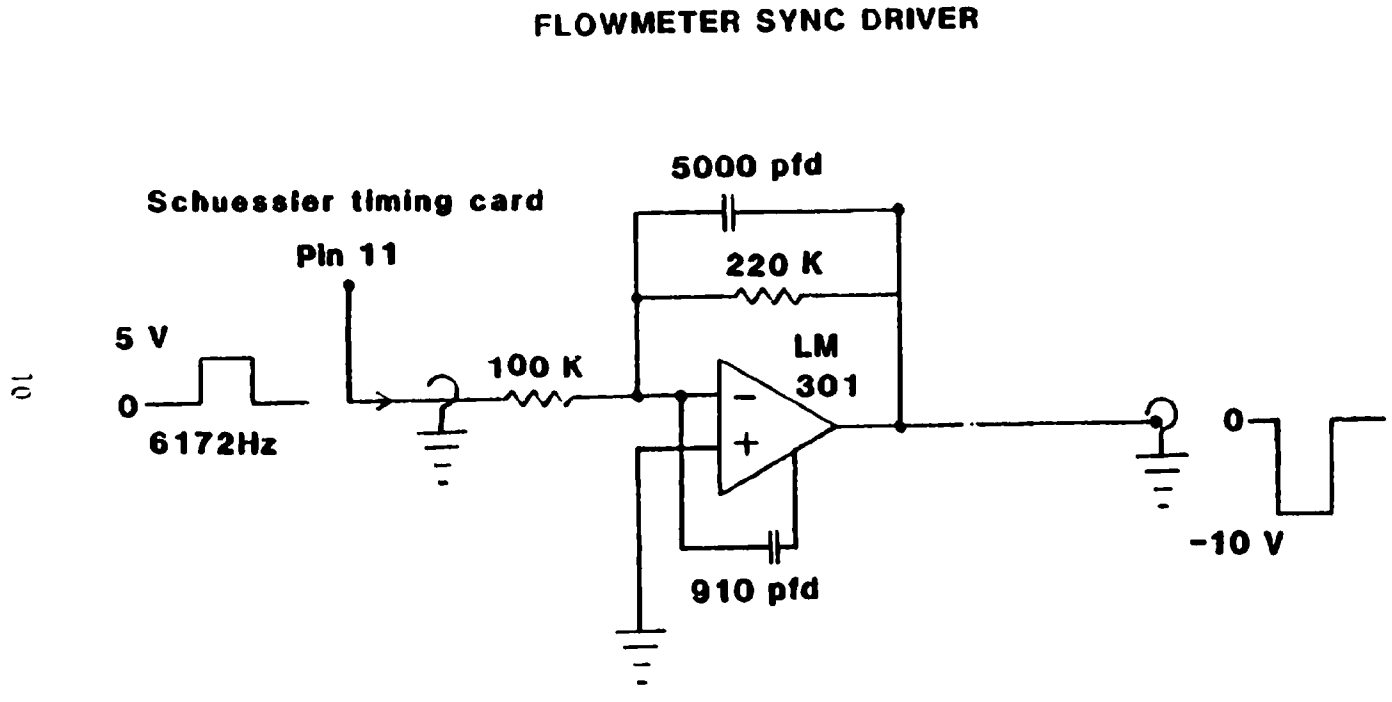


FIGURE A-2

Schematic of flow-meter synchronization driving circuit

The following assumptions about the ellipsoidal shell were made: 1) the measured minor and major axis diameters were assumed to represent the external diameter of the shell, 2) the measured anterior wall thickness was used as the dynamic shell thickness at the minor axis circumference, 3) the shell thickness at the base and apex was assumed to be 55% of the equatorial value (based on measurements on postmortem hearts). The dynamic internal volume of the shell was computed using the formula for a prolate ellipsoid:

$$V = \frac{\pi}{6} (b-2h)^2 (a-1.1h)$$

where b is the external minor axis diameter, h is the equatorial wall thickness, and a is the external major axis diameter. Stroke volume was computed as the change in internal shell volume during ejection. Stroke volume was also calculated by integration of the aortic flow curve assuming zero flow at end-diastole. The stroke volume measured with the flowmeter can also be compared to the data calculated from the dimension measurements.

Differentiation of the calculated volume curve can then be used to determine inflow and outflow from the left ventricle and again outflow can be compared to the electromagnetic flow trace. For left ventricular pressure-volume plots, left ventricular pressure was filtered in the same manner as that for volume and plotted against the corresponding value of left ventricular pressure.

More recently, an analog circuit has been constructed which computes left ventricular volume in real time during the experiment. The basis for the calculation is the same as that described above.

## EXPERIMENTAL PROTOCOL

On the day of the experiment, the animal was tranquilized with an intramuscular injection of Innovar Vet at 0.075 cc/kg. Piezoelectric manometer-tipped catheters (Millar PC 350, 5 French) were placed, under local anesthetic, in the right and left ventricles via small branches of a main femoral vein and artery, respectively. The arterial Millar gauge was used to calibrate the implanted Konigsberg gauge and then retracted into the aorta, just outside the aortic valve, to measure arterial pressure. The animal was maintained in a lightly tranquilized state for the duration of the experiment with serial injections of Innovar (0.5 cc/hr) administered through the right atrial cannula.

The measured physiological variables included aortic pressure and flow, left and right ventricular pressure and heart rate. In addition, measurements of left ventricular, major and minor axis dimensions and wall thickness have been added as described above. On-line, digitally calculated variables included beat-by-beat stroke volume, cardiac output, peripheral vascular resistance, maximum dp/dt, and the pressure difference from the aorta to the right atrium. Left ventricular volume is either calculated digitally off-line or on-line by an analog computer circuit.

### Procedure for autonomic blockade:

In order to delineate the neural and nonneural components of the measured cardiovascular responses to acceleration, a pharmacologically-induced total autonomic blockade was used to inhibit adrenergic and cholinergic activity at the effector site, thus removing normal reflex barostatic action.

The total autonomic blockade for the proposed study consisted of the alpha adrenergic blocker phenoxybenzamine (Dibenzyline) at 20 to 30 mg/kg administered

over an hour, beta blockade with propranolol (Inderal) at 1 to 2 mg/kg over approximately ten minutes, and cholinergic blockade with atropine (Atropine Sulphate) at 0.1 to 0.2 mg/kg over approximately five minutes. The efficacy of the blockade was tested and verified by a comparison of systemic responses to specific agonists given prior to blockade, following blockade and then again at the conclusion of the blocked acceleration sequence. These consisted of a 50 µg/kg bolus of phenylephrine (Neosynephrine) to test the alpha blockade and a 0.5 µg/kg bolus of isoproterenol (Isuprel) to test the beta blockade. If heart rate showed evidence of reflexive parasympathetic activity (i.e., a decrease following phenylephrine) the atropine dosage was supplemented.

Test Sequence:

The test sequence and experimental conditions were the same as in the previous years to allow for correlation of the experimental results. The sequence consists of $\pm$ 2 $g_z$ sinusoidal acceleration from 0.004 to 0.3 Hz starting at the low frequency and moving to the next higher frequency at 3 to 4 minute intervals without stopping the centrifuge. Previous years' studies indicated that this protocol allowed steady state conditions to develop more rapidly than did a protocol which stopped the centrifuge between frequencies. The sinusoidal series of tests was followed by a step input series consisting of a 3 minutes each from + 2 $g_z$ to - 2 $g_y$ to - 2 $g_z$ to + 2 $g_y$ to + 2 $g_z$ to - 2 $g_z$ to + 2 $g_z$ and back to - 2 $g_y$. The first 4 inputs were produced by 90° rotation of the platform while the centrifuge is producing 2 g radial acceleration. The last 2 inputs were produced by a 180° rotation each.

PRELIMINARY RESULTS

An example of left ventricular major and minor axes and wall thickness variations during several cardiac cycles is shown in Figure A-3. As described above, the three measurements were used to calculate the chamber volume variation during the cardiac cycle (lower trace). The data presented are from the off-line digital computations. The time derivative of the volume curve presented as the second trace of Figure A-4 shows the inflow to the left ventricle as positive values and the flow out of the chamber as negative values. Flow into the ventricle is seen to be biphasic with the primary peak due to left ventricular relaxation and the secondary peak due to the left atrial contraction.

The third and fourth traces of Figure A-4 are the flow out of the ventricle and that measured by an electromagnetic flow probe, respectively, and are shown for purposes of comparison. In Figure A-5, the stroke volume for two heart beats of another animal in the control state were calculated by taking the difference in the values of diastolic and systolic volumes. Stroke volume was also obtained by using the Trapezoidal rule to calculate the area under the aortic flow trace in Figure A-6. The values agreed to within 3%. The agreement is surprisingly good, but is expected to deteriorate as the data from more animals are analyzed.

Changes in left ventricular volume (from surgery to experiment to autopsy:

To date, crystals have been implanted to record heart size in 27 animals (16 normal and 11 cardiac denervated) whose body weights ranged from 17 to 26 kg. In the normal dogs, at surgery, major axis averaged 70.1 $\pm$ 1.6

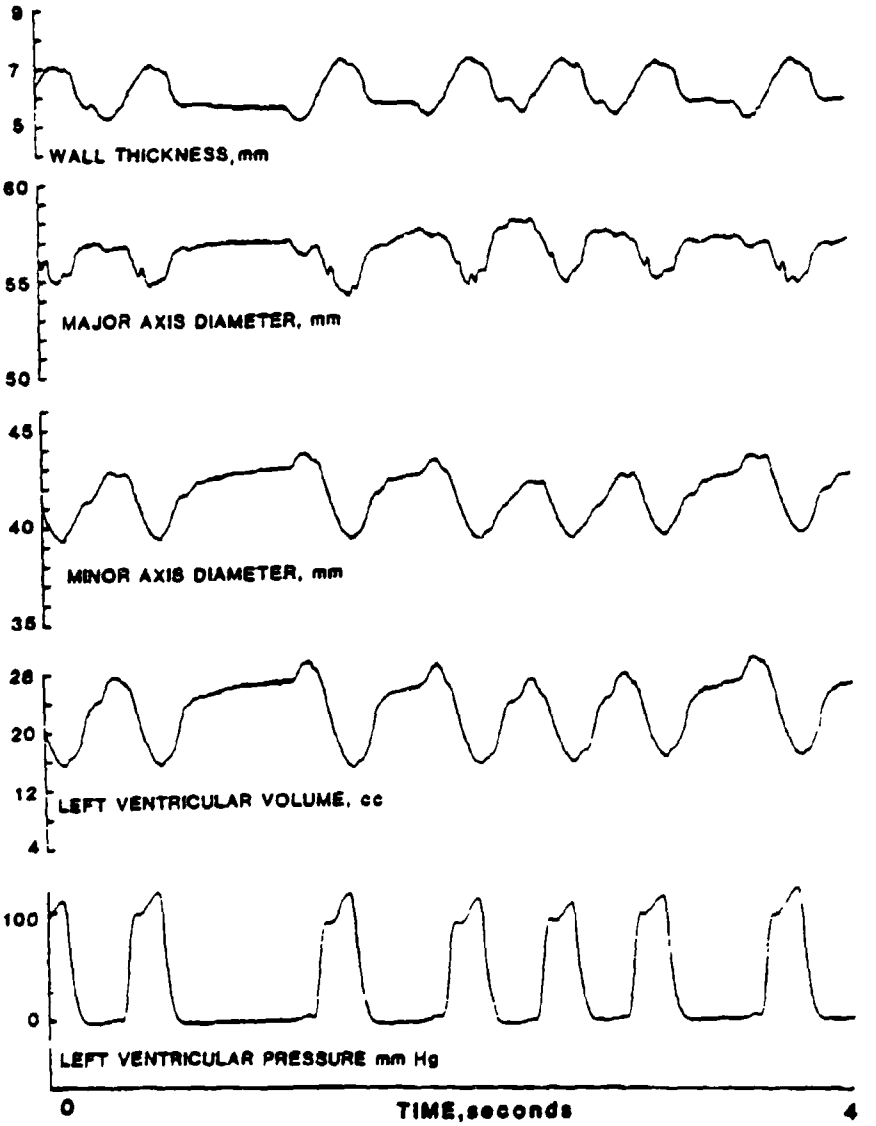


FIGURE A-3

Wall thickness, major and minor axis dimension, calculated left ventricular volume and left ventricular pressure for several heart beats from one dog in the control state

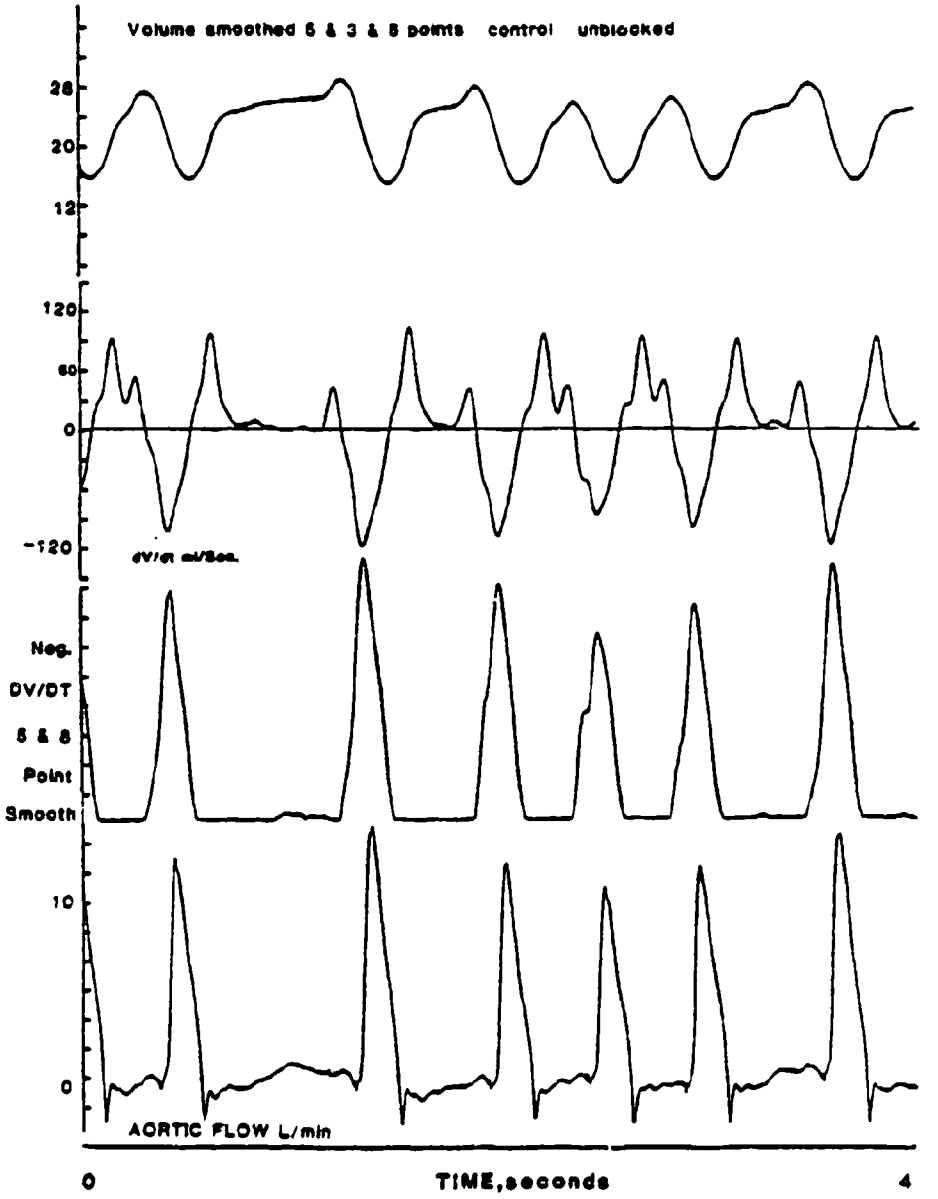


FIGURE A-4

Calculated, smoothed volume, its time derivative, the negative portion of the derivative and electromagnetic flow for the same animal during the same time period as FIGURE A-3

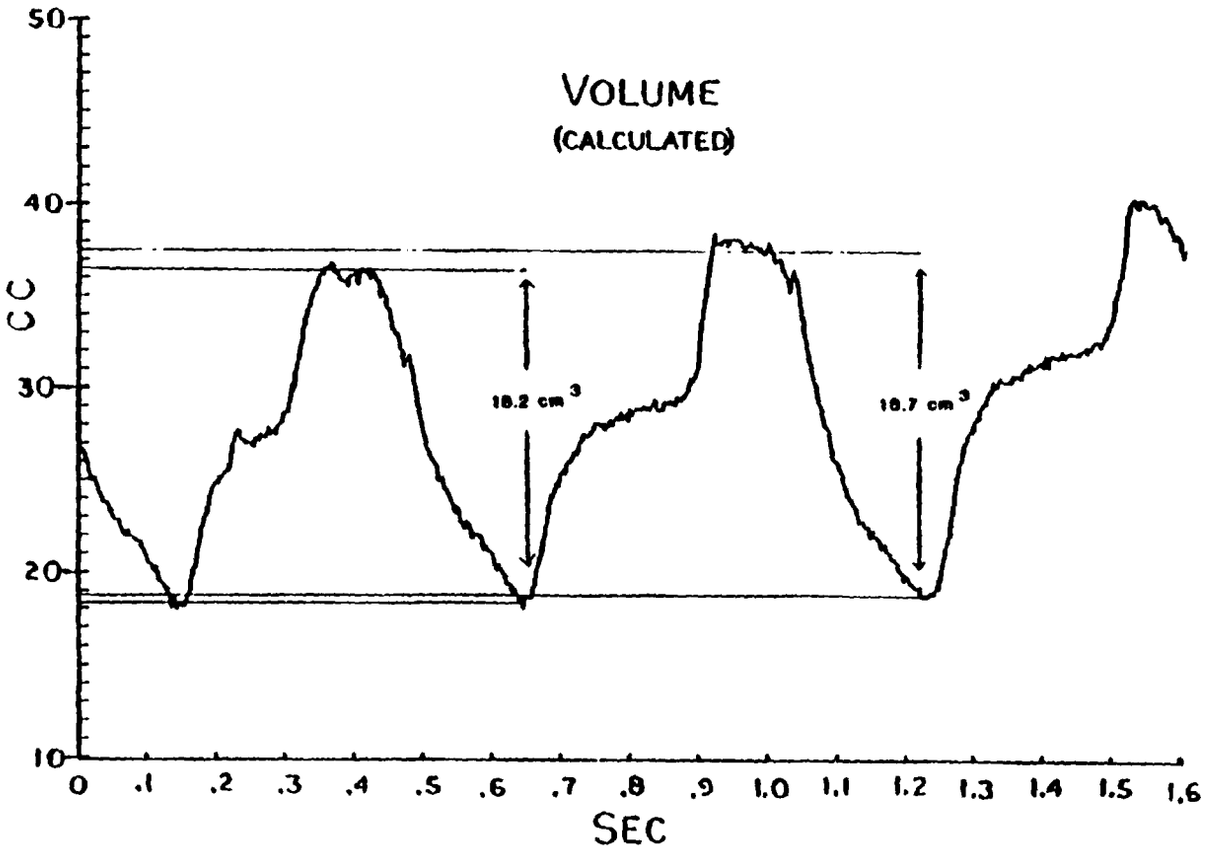


FIGURE A-5 Stroke volume as calculated from the difference in left ventricular end diastolic volume and end systolic volume for one animal in the control state

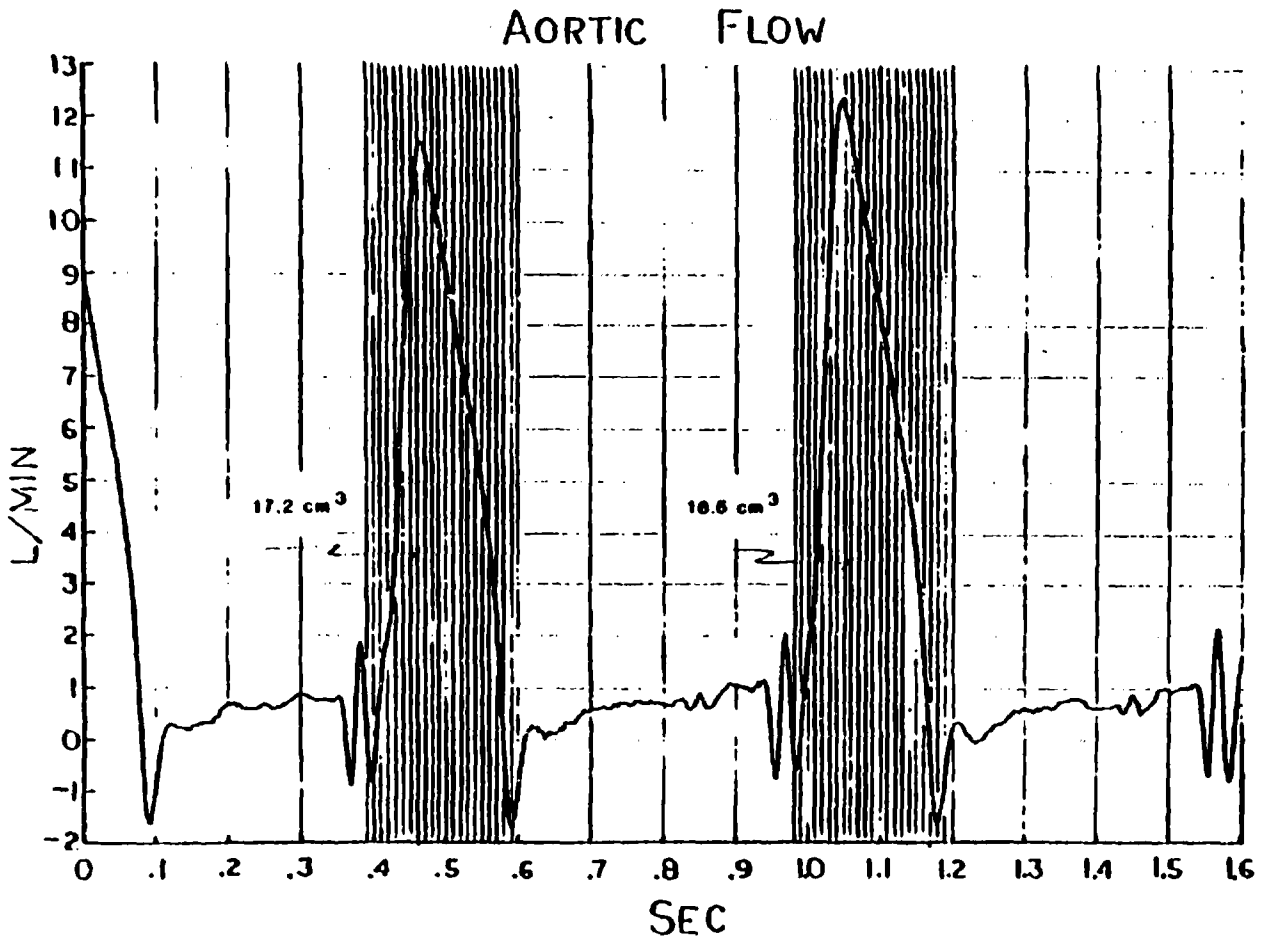


FIGURE A-6 Stroke volume as calculated from the electromagnetic flow trace for the same beats as those shown in FIGURE A-5

mm (S.E.M.), minor axis averaged 53.4 ± 1.1 mm, wall thickness averaged 12.6 ± 0.9 and calculated volumes averaged 25.0 ± 3.5 cc. For cardiac denervated dogs, following denervation surgery, major axis averaged 67.2 ± 1.6 mm, minor axis averaged 55.5 ± 2.3 mm, wall thickness averaged 12.3 ± 0.7 mm and calculated volumes averaged 31.0 ± 6.7 cc. At autopsy, the placement of the crystals was examined carefully, and in the case of major and minor axes, was found to be quite accurate; however, placement of the wall thickness crystals was often found to be inaccurate, and autopsy thickness was used to normalize actual volume calculations. Verifications of actual heart volumes by silastic casts in 10 animals at autopsy indicated a mean volume of 29.1 ± 1.5 cc in animals whose calculated autopsy volumes averaged 25.6 ± 4.8 cc. During experiments performed on these 10 animals, volumes in the control state averaged 29 ± 5 cc, generally increased with beta blockade (21 ± 7%), decreased slightly with the addition of cholinergic blockade and were slightly enlarged with the addition of α blockade, resulting in the largest control volume seen for the series. In the 11 cardiac denervated dogs, heart size was measured before and immediately after the denervation surgery (a 1 1/2 hour procedure). Heart size was found to generally increase with denervation from 27 ± 7 to 32 ± 7 cc.

A preliminary conclusion can be drawn for the data analysis to date: since the heart size was consistently greatest in the totally blocked control, the diminished output of the heart (stroke volume down by 1/2 from unblocked control) in this state can be attributed to blockade of autonomic efferent activity, rather than loss of plasma volume from the central circulation.

Sinusoidal Acceleration Response:

Seven normal and 5 cardiac denervated animals with instrumentation for recording left ventricular, right ventricular, and aortic arch pressure, aortic flow and left ventricular major, minor and wall thickness dimensions have been studied on the centrifuge. As with our past studies, the protocol consisted of 45 min. of sinusoidal $\pm$ 2 $g_z$ acceleration from 0.004 to 0.25 Hz followed by a series of 3 min. step-g inputs in the 1) normal or cardiac denervated animal, 2) beta blocked animal, 3) beta and cholinergically blocked animal and finally, 4) the totally blocked animal (beta, alpha and cholinergically blocked state). An example from one of these tests, 0.038 Hz, for one animal in the normal state, is shown in Figures A-7 and A-8 (the time scale has been expanded in two areas to show details of the traces). As in data reported previously for unblocked animals, mean aortic pressure is seen to oscillate with the acceleration, 130 mm Hg, at - 2 $g_z$ and 90 mm Hg at + 2 $g_z$ (trace 1, Figure A-7). This acceleration frequency is in the region where we have previously noted the largest oscillations in pressure for the frequency range studies. As in our other normal animals at this frequency, peripheral resistance appears to be changing in a totally counterproductive manner (trace 6, Figure A-7), maximum values with - 2 $g_z$ and minimum values with + 2 $g_z$ while stroke volume, as usual, behaved quite hydraulically (trace 4, Figure A-7). It is in this region that heart rate is required to compensate for the counterproductive effects of peripheral resistance and stroke volume, and therefore undergoes large oscillations (trace 3, Figure A-7) to maintain cardiac output at a level as constant as possible (trace 5, Figure A-7). To determine the amount of blood returned to the ventricle and its subsequent ejection in the acceleration environment, volume is calculated on a 6 msec

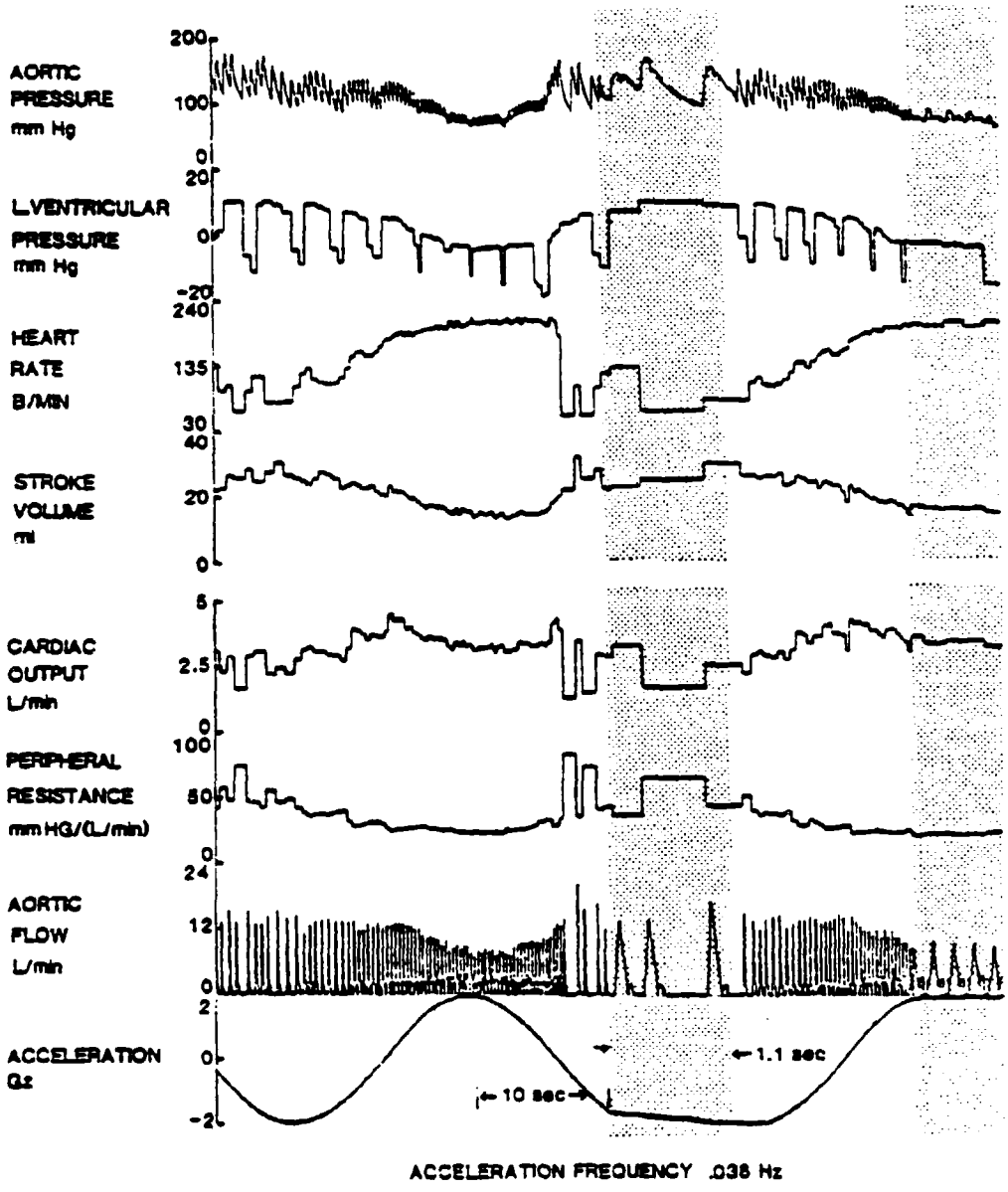


FIGURE A-7 Routine analog and digitally calculated variables for one animal during 2 $g_z$ sinusoidal acceleration at 0.038 Hz. An expanded time scale is indicated by the stipled area.

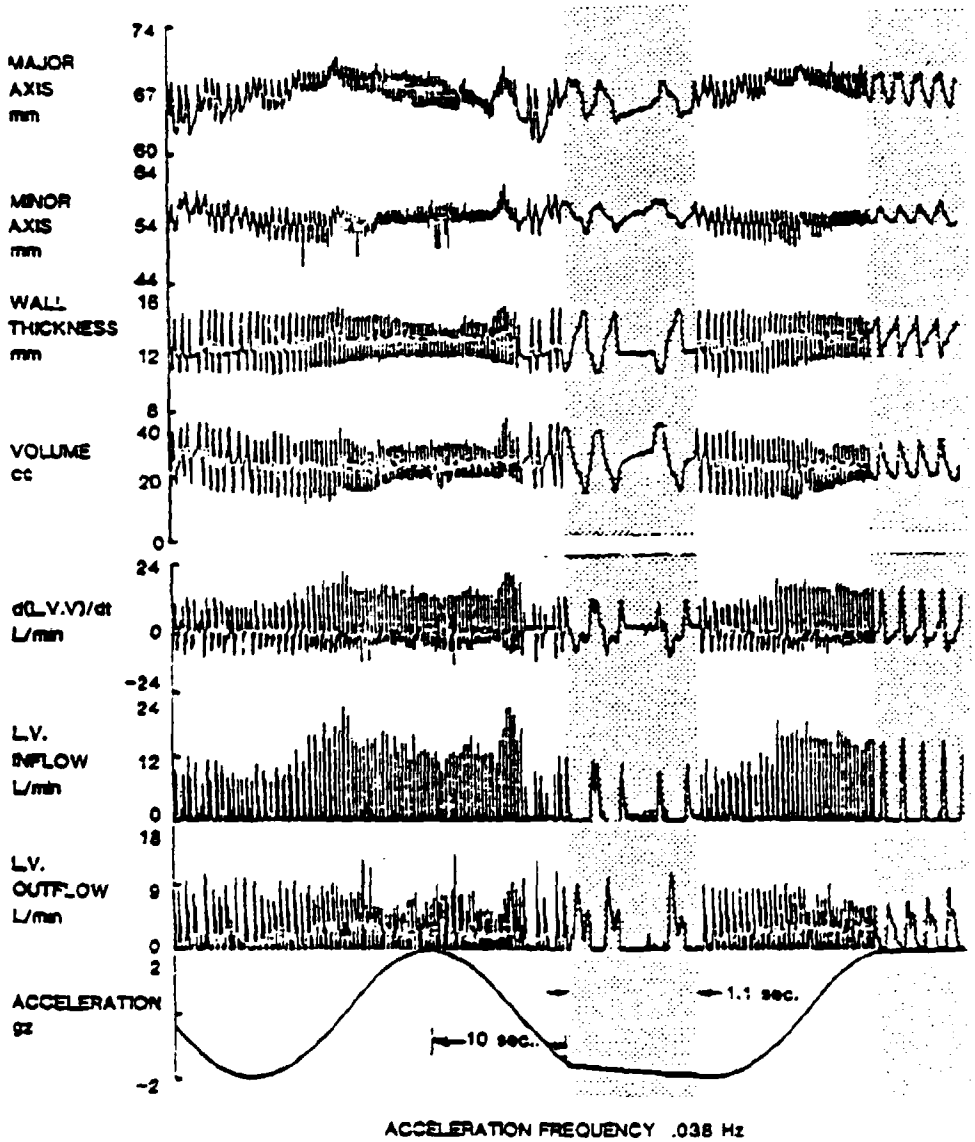


FIGURE A-8 Cardiac dimension and calculated dimension parameters for the same animal, during the same time period as that of FIGURE A-7

interval and displayed continuously (trace 4, Figure A-8). The diastolic volume of the left ventricle, the upper envelope of the trace, indicates that the volume returned to the ventricle is maximal with - 2 $g_z$ and minimal with + 2 $g_z$. The volume remaining in the ventricle after ejection, the lower envelope of the trace, was minimal with - 2 $g_z$ and maximal with + 2 $g_z$. The difference in the maximum and minimum values within a beat, the stroke volume of the left ventricle, is therefore maximal with - 2 $g_z$ and minimal with + 2 $g_z$. A comparison of actual values of stroke volume as computed from the volume trace and the electromagnetic trace indicates a stroke volume of 22 ml (electromagnetic) and 16 ml (volume calculation) at - 2 $g_z$ and a volume of 11 ml (electromagnetic) and 8 ml (volume calculation) at + 2 $g_z$. This validation of the stroke volume calculation gives us confidence in interpreting the volume trace in our experimental environment and holds promise for answering the questions we have previously asked concerning the ability of cardiac denervated animals to maintain stroke volume in the + 2 $g_z$ environment. (Data analysis is currently being conducted).

Response to rapid onset acceleration:

An example of the response of one animal in the normal state to a sudden + 2 $g_z$ acceleration loading is shown in Figures A-9 and A-10. The time scale of the trace has been expanded during the rapid rise in acceleration and later during a more steady state period. Of particular note is the increase in left ventricular end diastolic volume during the initial phase of the + 2 $g_z$ onset, followed by a decrease after steady state conditions were reached. The initial increase in left ventricular end diastolic volume is most likely due to volume shifts from the pulmonary circulation due to the

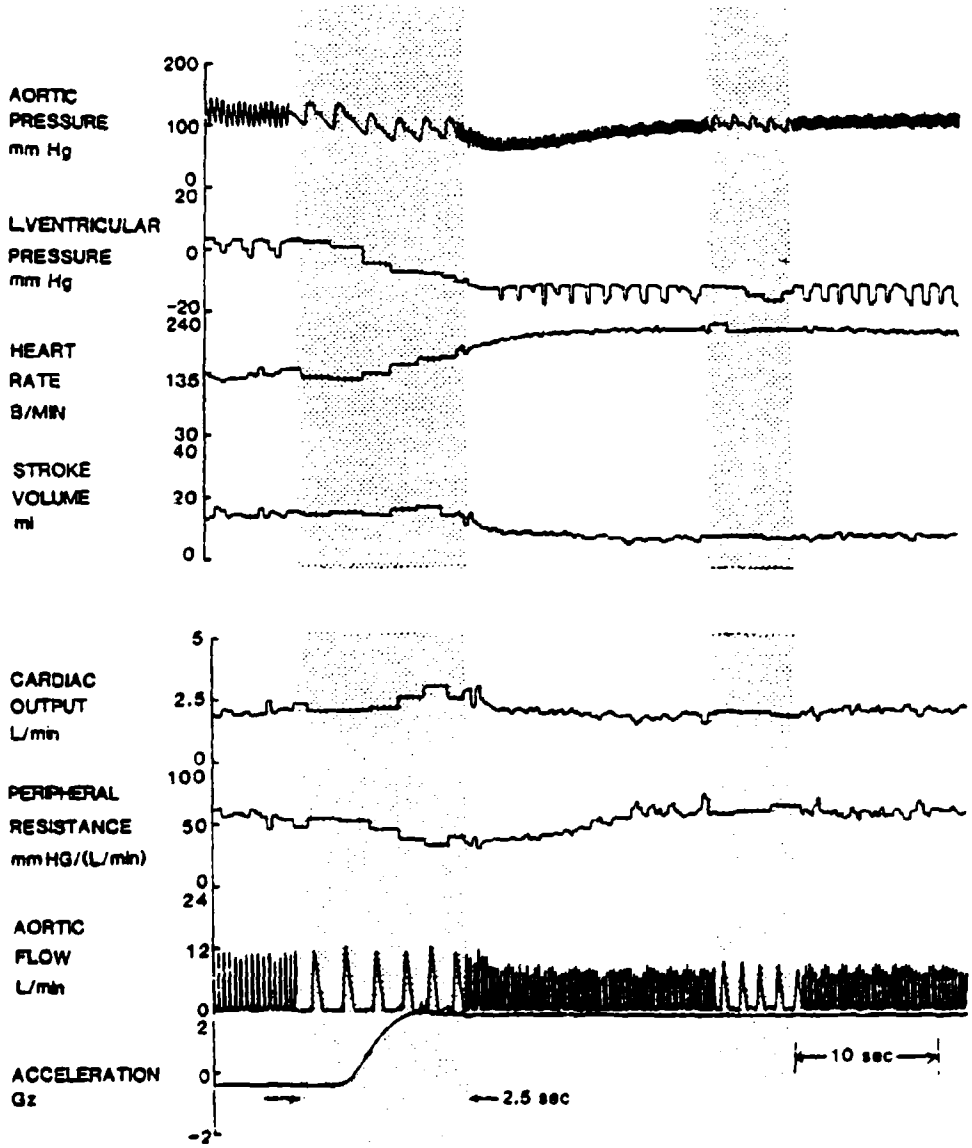


FIGURE A-9 Routine analog and digitally calculated variables for the same animal as FIGURES A-7 and A-8 during a +2 $g_z$ step input. An expanded time scale is indicated by the stipled area.

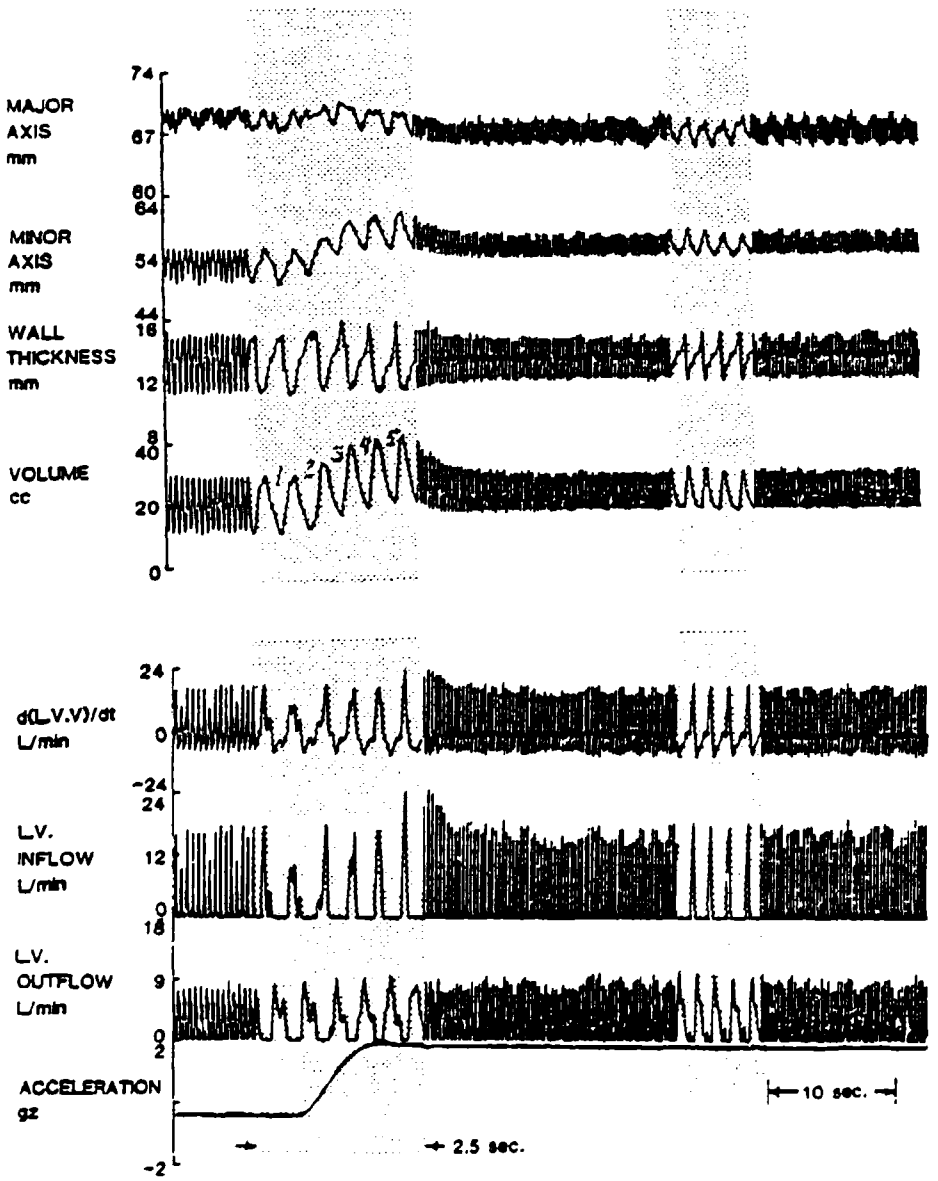


FIGURE A-10 Cardiac dimensions and calculated dimension parameters for the same animal, during the same time period as that of FIGURE A-9

+ 2 $g_z$ load. This increase in end diastolic volume, however, is not associated with an increase in stroke volume and, most interestingly, it occurs during a time when left ventricular diastolic pressure is falling (it must be remembered that the pressure gradient between the left ventricle and the pulmonary vein is actually responsible for left ventricular inflow). This response is also shown by the left ventricular volume vs. pressure curve of Figure A-11. It is suspected that these initial responses (first 2 or 3 beats after the initiation of the acceleration) most likely reflect passive hydraulic mechanisms because of the 1-3 sec. over which they occur. However, these observations are extremely preliminary and must await further studies.

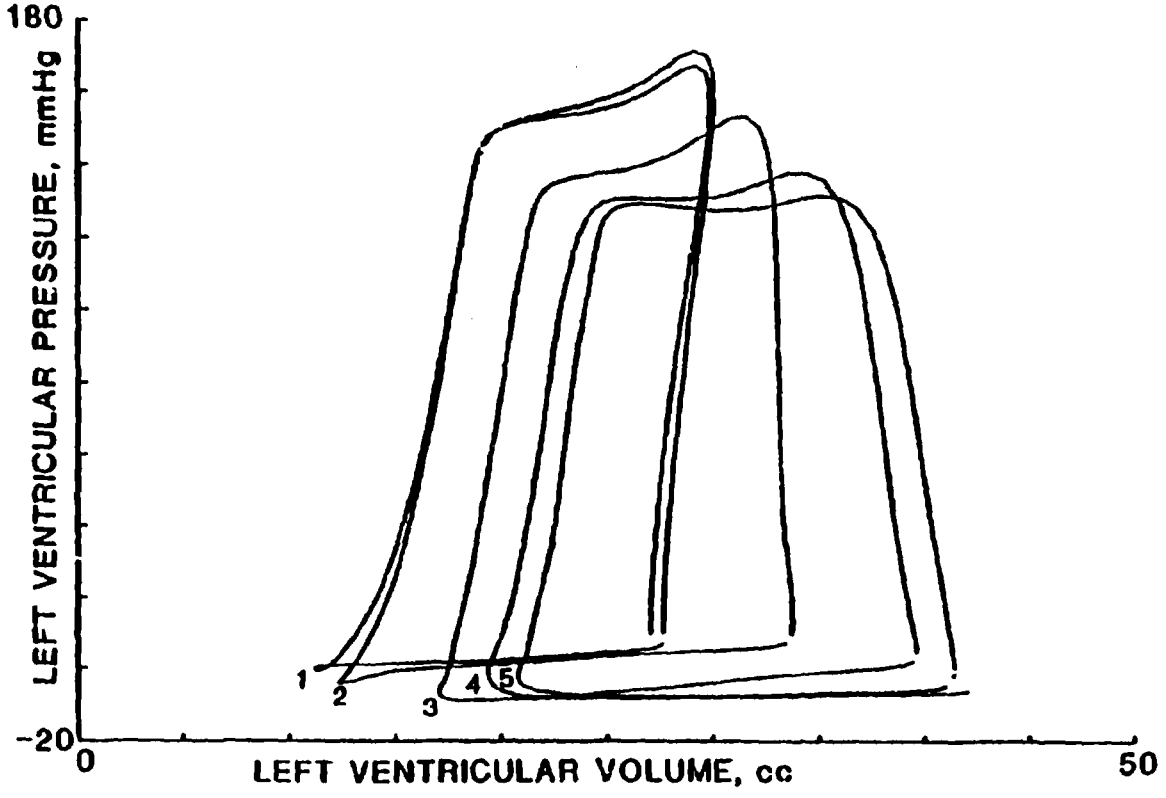


FIGURE A-11 Left ventricular volume as a function of left ventricular pressure for 5 heart beats occurring during the +2 $g_z$ onset of FIGURE A-10

## REFERENCES (Section A)

1. Rankin, J. S., P. A. McHale, C. E. Arentzen, D. Ling, J. C. Greenfield and R. W. Anderson. Circ. Res. 39(3): 304-313, 1976.

## B. NEUROHORMONAL COMPONENTS OF AN ACCELERATION-INDUCED PRESSOR RESPONSE IN NORMAL AND CARDIAC DENERVATED CONSCIOUS DOGS

An increase in aortic pressure has been observed in response to stresses which tend to pool blood in the lower body (2, 4), the upper body (3) and oscillatory stress which would be expected to exert a net zero pooling effect (5, 7). A major research effort has been conducted to elucidate the pressor components of the response to lower body pooling and the associated reduction of plasma volume and stroke volume observed in orthostasis (2), lower body negative pressure (2) and elevated $+g_z$ gravitational fields (2, 8, 10). Dissection of this response has indicated activation of a generalized sympathetic response (2, 10) resulting in increases in both heart rate (2, 10) and peripheral vascular resistance (2, 8, 10). Reflex, anti-hypotensive buffering agents which have been detected include neurally-mediated release of norepinephrine (8, 10) and neurosecretory release of a) epinephrine and norepinephrine from the adrenal medulla (14), b) plasma renin from the kidney and other sites (17, 18) and c) vasopressin from the neurohypophysis (1). Pressure maintenance in the above studies was partially attributed to autonomic neural effector activity. Our interest in the phenomena developed when we observed a greater pressor response to acceleration stress in the presence of a well documented blockade of autonomic effector sites than was observed in nonblocked, acceleration stressed animals.

For the past several years, we have observed that whole body, low frequency, sinusoidal acceleration routinely produced an increase in aortic pressure in normal and cardiac denervated, chronically instrumented, Innovar-sedated dogs. Elevated mean arterial pressure was evident within the first few minutes of centrifugation, persisted throughout the 30 to 40 minutes of the test period, appeared to be independent of the frequency of acceleration, and remained for

several (5 to 10) minutes following the end of the test period. In addition, it appeared to be independent of both cardiac innervation, particularly right heart afferent information, and of autonomic effector activity, since it was present to a greater extent in the same dogs following pharmacological blockade of sympathetic $\alpha$, $\beta$ and cholinergic activity. The magnitude of the response increased aortic pressure by approximately 15 mm Hg in reflexive dogs whether normal or cardiac denervated and more than double that amount in the same dogs in the nonreflexive state.

A separate study was therefore initiated to quantify the response in terms of an increase in plasma volume, peripheral resistance, stroke volume or heart rate and its correlation with various neural and hormonal mechanisms. The animals used in this study were a random sampling of the normal and cardiac denervated dogs undergoing our normal acceleration protocol. For details, see Experimental Protocol of Section A.

METHODS

Surgical:

a. Cardiac denervation. In sixteen of the thirty eight dogs used in this study, the method of Randall (11) was used for denervation of the heart prior to implantation of instrumentation detailed below. The efficacy of the denervation was confirmed prior to chest closure by demonstrating the complete absence of change in atrial and ventricular contractile force and heart rate during stimulation of the left and right thoracic vagi and left and right stellate ganglion, all of which could be visualized through the left thoracic incision used in the implant procedure. On the day of the experiment, denervation was confirmed by the absence of reflex cardiac response to right atrial injections of 8 ug/kg of nitroglycerine (Nitrostat) and 50 ug/kg of phenylephrine (neosynephrine).

b. Implant procedures. Both normal and cardiac denervated dogs underwent the same implant procedure. Each was anesthetized with sodium thiopental (dosage) and was prepared for sterile surgery. A thoracotomy was performed at L-4 and a pressure gauge (Konigsberg Instruments, model P19) was placed through the apex of the heart into the left ventricular chamber. An electromagnetic flow cuff (Zepeda Instruments) was placed on the ascending aorta and a polyvinyl cannula was placed through the right atrial appendage. Three weeks later, on the day of study, manometer-tipped catheters (Millar PC 350, 5 French) were placed, under Innovar tranquilization (1.5 ml/20 kg, IM) and local anesthetic, in the right and left ventricles of the heart via small branches of the femoral vein and artery. The arterial gauge was used to calibrate the implanted gauge and was then retracted into the aorta, just outside the valve, to record arterial pressure for the remainder of the study.

c. Postoperative care. The surgery, recovery and experiments were conducted in accordance with those procedures outlined in the "Guiding Principles in the Care and Use of Animals". Each animal was allowed a minimum of three weeks of postoperative recovery before studies were begun. Postoperative management included antibiotic coverage and particular attention to body temperature and nutritional and hemotologic factors. Studies were not performed unless these factors were stable and within a range considered to indicate a satisfactory state of health.

Experimental Protocol and Blood Sampling Techniques:

Right atrial blood samples were withdrawn from twenty seven animals (16 normal and 11 denervated) within 3 minutes before and within 3 minutes following, 30 minutes of sinusoidal acceleration stress. Pre and post

acceleration samples were withdrawn in both the 'reflexive' state and following total autonomic blockade, detailed below. In addition to hematocrit, each sample was analyzed for at least two of the following substances: plasma renin activity, plasma osmolality and arginine vasopressin activity (ADH), plasma catecholamines and/or plasma volume. Subsequent analysis of these samples was performed by various laboratories chosen for their expertise in the analyses to be performed.

a. Plasma renin activity. Plasma renin activity was determined by Dr. Theodore Kotchin of the University of Kentucky, Department of Medicine.

b. Plasma arginine vasopressin activity and osmolality. Was determined by Dr. Gary Robertson of the University of Chicago, Department of Endocrinology.

c. Plasma norepinephrine and epinephrine activity. Was determined by Dr. Michael Ziegler of the University of Texas, Department of Surgery and Internal Medicine.

d. Plasma volume and hematocrit. Plasma volume was determined by the $RISA^{131}$ technique through the Department of Nuclear Medicine, University of Kentucky and hematocrit was done in our own laboratory.

Total Autonomic Efferent Blockade :

Blockade of autonomic effector activity was established in each animal in the same manner. Beta adrenergic activity was recorded in response to a 10 µg injection of isoproterenol (Isuprel), followed by activation of alpha adrenergic response to a 50 µg/kg injection of phenylephrine (Neosynephrine). Beta adrenergic blockade was then established with a 1 mg/kg infusion of propranolol (Inderal) the dosage used being that required to block any cardiovascular response to a repeated injection of Isuprel.

Next, muscarinic cholinergic blockade was established with a .1 mg/kg infusion of atropine sulfate. Any change in heart rate with the beta blockade already established was then used as an indicator of diminished cholinergic blockade. Finally, alpha adrenergic blockade was established with an hour-long infusion of 30 mg/kg phenoxybenzamine (Dibenzyline). At the end of this infusion period, second dosages of the beta and cholinergic blockers were administered, the absence of response to the alpha and beta agonists was verified, blood samples were withdrawn, and 30 to 40 min. of acceleration was begun. Within 3 min. of the cessation of acceleration, blood samples were again withdrawn and the blockades were again verified. In some animals, the beta blockade was found to be compromised at the end of the run and the data from these animals was not included in the following hemodynamic results.

DATA ACQUISITION

An on-line digital computer sampled all signals at 2-ms intervals during systole and at 8-ms intervals during diastolic. Aortic and left and right ventricular pressures were analyzed for peak systolic, mean diastolic ard pulse pressures. The program integrated the aortic flow signal after determining flow zero and eliminating pacing and/or ECG artifacts, producing a correct value of stroke volume (ml/beat). Stroke volume was then multiplied by the preceding heart rate (beats/min) and converted to an analog signal to give a one-beat-delayed, beat-by-beat strip-chart recording of cardiac output (ml/min). Using values stored from an individual beat, the peripheral vascular resistance was calculated with the use of the pressure difference from the aortic valve to the tricuspid valve (mean aortic pressure -- diastolic right ventricular pressure), divided by cardiac output (stroke

volume X heart rate). Each computed variable was converted to an analog signal, sent to analog tape for later analysis, to the strip-chart recorder for continuous monitoring and to digital tape for summary over each control, test and recovery period.

Statistical Analysis:

The change in aortic blood pressure in each experimental condition (pre and post acceleration) for both the normal and cardiac denervated dogs before and after autonomic blockade was tested statistically with a one-way analysis of variance and subsequent t-test for related measures to indicate which means were significantly different. Likewise, changes in heart rate, stroke volume, cardiac output, peripheral resistance, hematocrit, plasma renin activity, plasma levels of vasopressin and plasma levels of catecholamines were tested for significance using the same analysis.

## RESULTS

Hemodynamic:

Response of a typical unblocked dog to 30 min. of sinusoidal acceleration are shown in Figure B-1a and responses of the same dog following autonomic blockade are shown in Figure B-1b.

The four segments shown in each figure are from (1) preacceleration control, (2) onset of acceleration, (3) 30 min. later during the final acceleration test, and (4) immediately post acceleration. Blood sampling was done during the first and last periods, indicated by arrows. In this animal, in the unblocked state (Figure B-1a), aortic blood pressure rises from a mean of 93 mm Hg preacceleration to 120 mm Hg post acceleration via an increase in both mean heart rate and stroke volume. In the same animal in the autonomically blocked state (Figure B-1b) pressure rises from a preacceleration mean

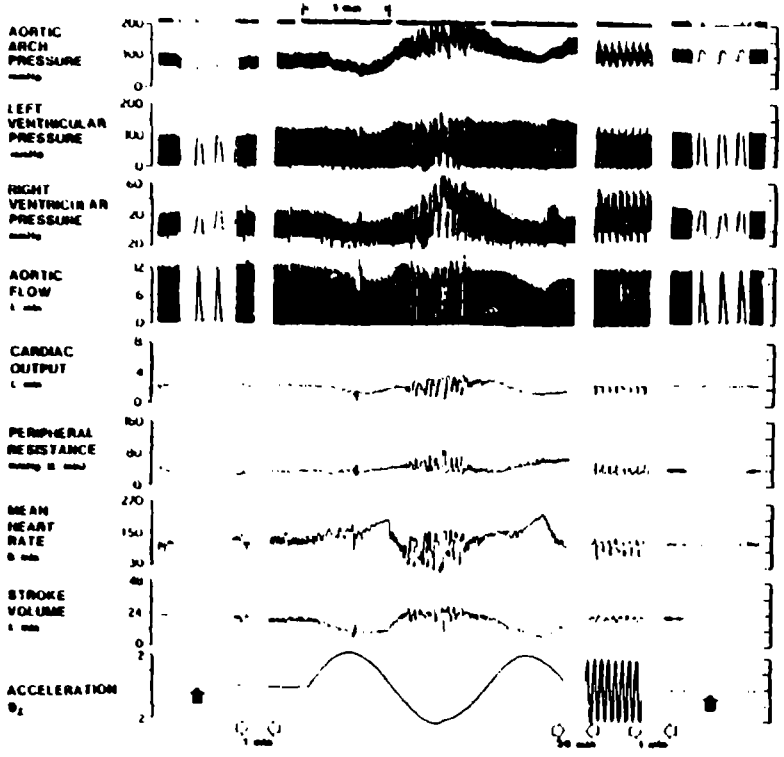


FIG. B-1a Response of an unblocked, normal dog to approximately 30 min. of + 2 $g_z$ acceleration stress. Hemodynamic and hemotologic data were taken at times indicated by black arrows

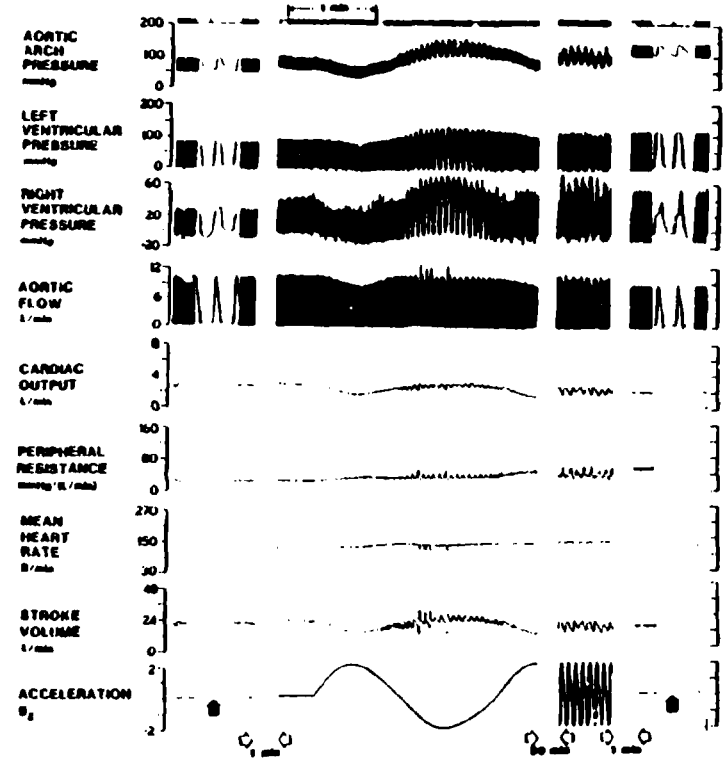


FIG. B-1b Response of same dog following total autonomic effector blockade

of 70 mm Hg to a post acceleration mean of 140 mm Hg via an increase in peripheral vascular resistance with no increase in either stroke volume or heart rate. Verification of autonomic blockade immediately followed the preacceleration blood sample, with no response to either isoproterenol or phenylephrine.

Hemodynamic results from the group of animals taken at the blood sampling (3 min. pre and post acceleration) indicated a group response which was quite similar to that of the single animal shown, and appeared to be fairly independent of cardiac innervation. (Figures B-2a and B-2b respectively). Normal animals (Figure B-2a) increased aortic pressure by 16 mm Hg in the unblocked state and by 48 mm Hg in the autonomically blocked state, both increases were significant. The pressor response in these animals was due to a significant increase of 0.5 L/min in cardiac output in the unblocked state and a significant increase of 19 mm Hg (L/min) in peripheral resistance in the autonomically blocked state. Furthermore, the cardiac output increase was due to a significant increase of 23 b/min in heart rate with no change in stroke volume.

The group of cardiac denervated animals (Figure B-2b) increased aortic pressure by 12 mm Hg in the unblocked state and by 28 mm Hg in the unblocked state, again both increases were significant. The pressor response in these animals in the unblocked state was due to a nonsignificant increase in both peripheral resistance and heart rate, while the totally blocked pressor response was due to a significant increase in peripheral resistance. Autonomic blockade produced a significant lowering of aortic pressure in normal animals via a significant decrease in stroke volume and a nonsignificant decrease in peripheral resistance. A few significant differences were noted between normal and cardiac denervated animals in this study: (1) totally

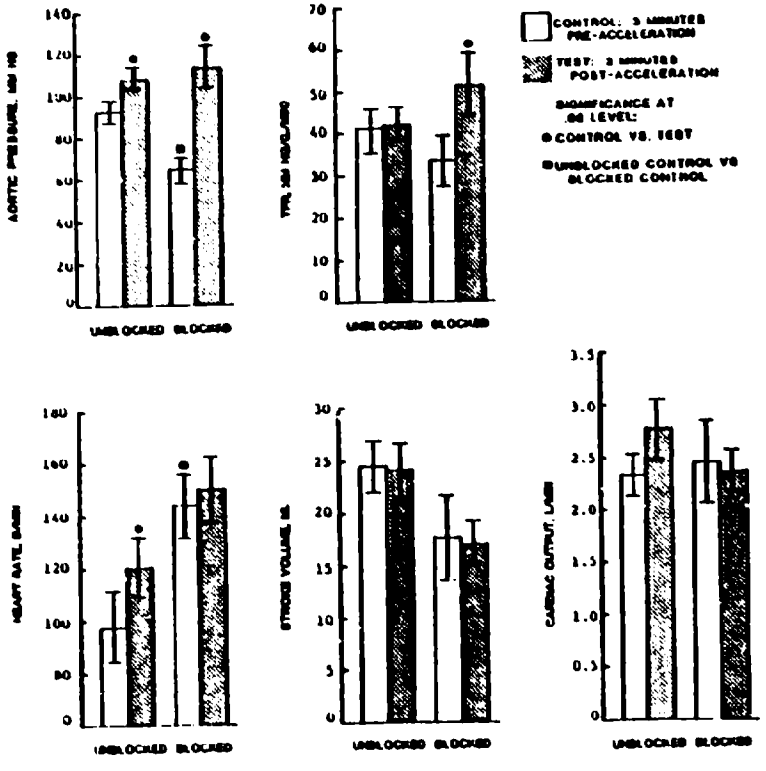


FIG. B-2a Mean ± S.E.M. responses to acceleration from a group of 8 normal animals. Data was taken before and after acceleration in each animal before and after total autonomic effector blockade.

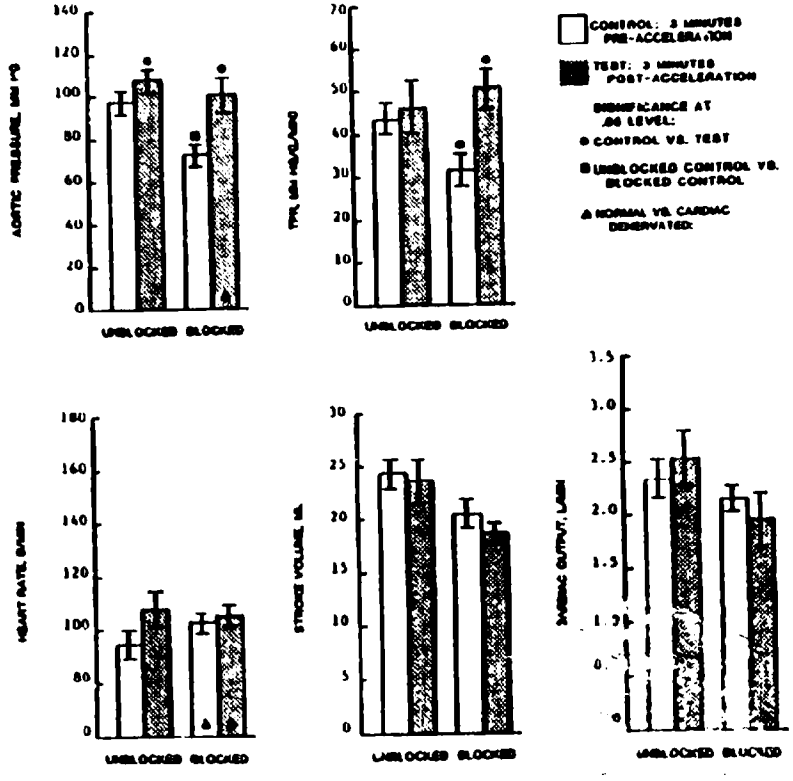


FIG. B-2b Mean ± S.E.M. responses to acceleration from a group of 8 cardiac denervated animals. Data was taken before and after acceleration in each animal before and after total autonomic effector blockade.

blocked heart rates were significantly lower in cardiac denervated animals both pre and post acceleration and (2) totally blocked, post-acceleration aortic pressure was significantly lower in cardiac denervated dogs as compared to normal dogs due to the lower heart rate of these animals. It was interesting to note that cardiac denervated dogs did not drop stroke volume with autonomic blockade to the extent that normally innervated dogs did.

Neurohumoral:

The neurohumoral responses of a group made up of both normal and cardiac denervated animals are shown in Figure B-3. The results from the two groups were pooled since, excepting control values of plasma volumes, and plasma renin activity, there was no apparent difference in the response of the two groups.

a. Plasma volume as indicated by $RISA^{131}$ or hematocrit changes. In contrast to the other variables measured, plasma volume did appear to change with the animal preparation (normal vs. cardiac denervated). Results from the $RISA^{131}$ and the Evans blue determinations indicated plasma volumes of 1063 $\pm$ 205 ml (S.E.M.) in 5 normal animals with plasma volumes of 765 $\pm$ 86 ml (S.E.M.) in 4 cardiac denervated animals. Preacceleration control values of hematocrit were also elevated in 7 cardiac denervated (39.9 $\pm$ 1.5) as compared to 9 normal (35.1 $\pm$ 1.3) dogs.

Acceleration-induced changes in plasma volume as indicated by serial determinations following the original $RISA^{131}$ injection were not consistent in either normal or cardiac denervated dogs. However, plasma volume changes as indicated by hematocrit (Figure 3a) were evident with each acceleration test in both normal and cardiac denervated dogs. Since hematocrit increased significantly with acceleration indicating, if not negating, a decrease in plasma volume, other variables were sought to explain the acceleration-induced pressor effect observed.

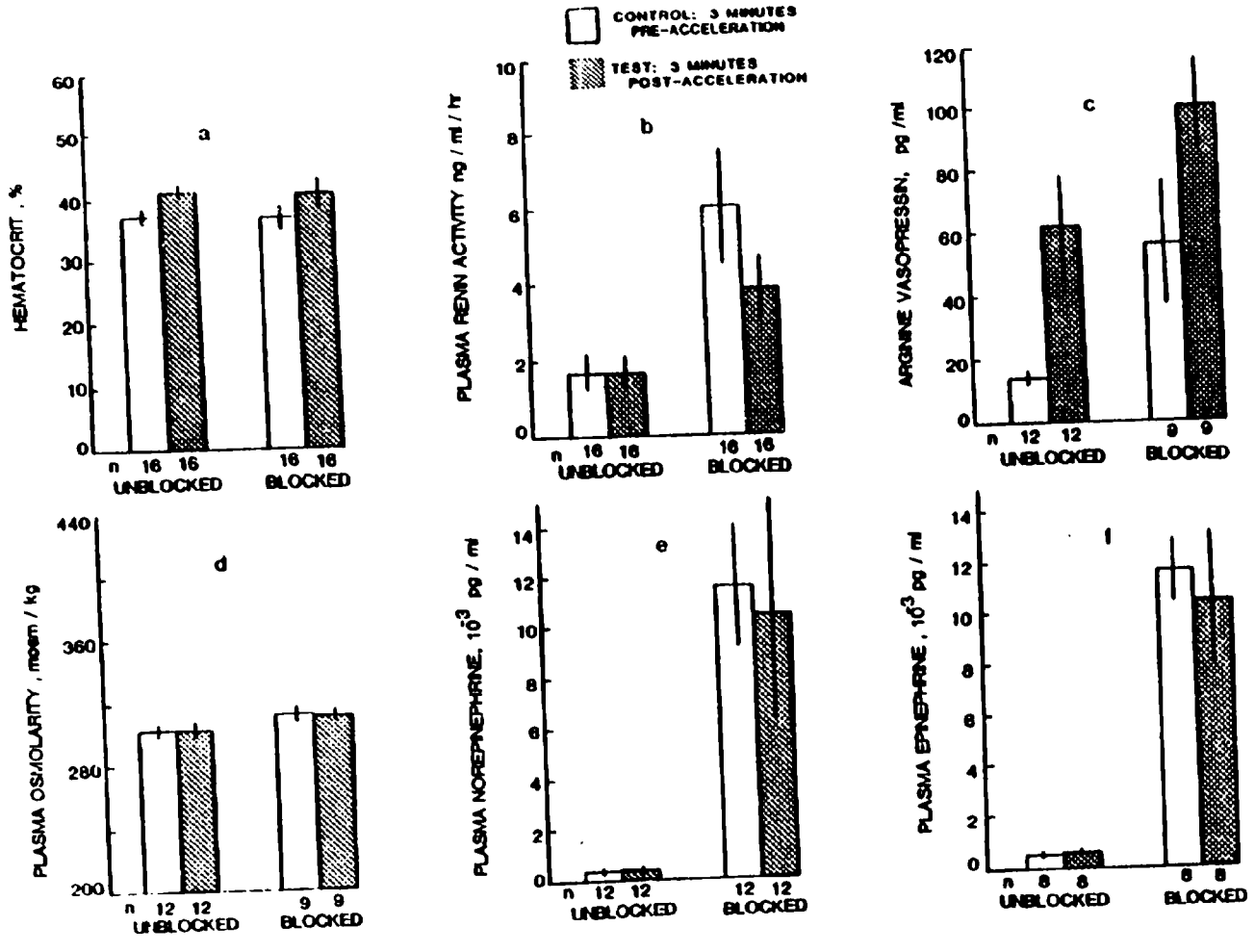


FIG. B-3 Neurohumoral responses to ~30 minutes of $\pm 2$ $g_z$ sinusoidal acceleration stress in a group of animals before (unblocked) and after (blocked) total autonomic effector blockade

b. Plasma renin activity. Plasma renin activity was measured in 12 normal dogs and 6 cardiac denervated dogs (Figure B-3b) in each of the four experimental conditions. Results of these measurements (Figure B-3b) indicated:

1) Two dogs (1 normal and 1 cardiac denervated) had extremely high (61 ng/ml/hr and 100 ng/ml/hr - respectively) control values and were excluded from further analysis.
2) All other dogs (11 normal at 2.3 $\pm$ 0.6 ng/ml/hr or 5 cardiac denervated at 0.5 $\pm$ 0.1 ng/ml/hr) had low values of plasma renin activity, perhaps due to their sedated but not anesthetized state.
3) Acceleration produced no consistent or significant changes in plasma renin activity in either the reflexive or nonreflexive state in either normal or cardiac denervated dogs.
4) Total autonomic blockade significantly increased (1.7 $\pm$ 0.5 ng/ml/hr to 6.0 $\pm$ 1.6 ng/ml/hr) circulating levels of plasma renin activity in the group of 16 dogs (14 increased, 2 decreased).

c. Plasma arginine vasopressin (ADH) and osmolarity activity. Plasma osmolarity and corresponding levels of ADH were measured in 10 normal dogs and 8 cardiac denervated dogs. In some of these animals, a strong and consistent pattern of ADH activity that was not reflected in osmolarity changes was noted, and this data will be presented here along with the criteria used to delete the other animals' responses. In the unblocked case, the criteria used was the control level of circulating ADH in which only those animals who

had < 30 pg/ml control values were included. In the totally blocked case, several animals were found to not exhibit an effective β adrenergic blockade at the end of the run, and these animals were deleted from this group. In this case, in contrast to the other variables reported in this study, there is a different group of animals in the unblocked and the totally blocked groups, but the preacceleration to post acceleration subjects in each group were the same.

Results from 12 unblocked animals (7 normal and 5 cardiac denervated) and from 9 totally blocked animals (6 normal and 3 cardiac denervated) who met the above criteria are shown in Figure B-3c. The normal and cardiac denervated results were combined since a comparison showed no difference in either unblocked control (normals: 14.3 $\pm$ 2.8, cardiac denervateds: 13.8 $\pm$ 4.8 pg/ml) or unblocked recovery (normals: 62 $\pm$ 15.8, cardiac denervateds: 69.6 $\pm$ 26.3 pg/ml) values of ADH. Several other observations were made however, concerning the 18 total animals in this group, their pressor and ADH responses to centrifugation.

There were 8 female and 10 male dogs in this section of the study and it was found that, in the unblocked case, all 10 of the male dogs had a marked pressor response (the group mean increased 19 $\pm$ 3 mm Hg) while four of the females increased and the other four decreased pressure (the group mean increased 2 $\pm$ 4 mm Hg). Additionally, 9 of the 10 males increased circulating ADH levels (group mean increased 33.2 $\pm$ 10.5 pg/ml) while 4 females increased and the other 4 decreased ADH levels (group mean increased 13.2 $\pm$ 4.7 pg/ml). Additionally, 4 of the 5 animals who had abnormally high (> 30 pg/ml) levels of ADH in the unblocked control state were female. Finally, autonomic blockade appeared to remove male-female differences in both pressor and ADH responses to centrifugation.

Plasma osmolarity was measured in conjunction with plasma ADH for each of the 18 animals in this part of the study. Results are shown in Figure B-3d for the same animals for which ADH activity is shown; 12 unblocked and 9 totally blocked subjects. Changes in plasma osmolarity could not be used to predict changes in ADH in this study, nor was there any predictable response in osmolarity to centrifugation. The one change in osmolarity which was predictable was an increase in response to the establishment of autonomic blockade.

From this section of the study, several guarded observations can be made:

1) Unblocked animals, either normal or cardiac denervated, who have reasonably low control levels of ADH can be expected to increase ADH in response to centrifugation, particularly if the subject is male.
2) Establishment of total autonomic effector blockade can be expected to increase plasma osmolarity in all animals and plasma ADH in those animals who had reasonably low control levels of ADH.
3) Centrifugation can be expected to increase ADH in animals who have well established autonomic effector blockades.
4) The stimulus to increase ADH levels with centrifugation was not derived from plasma osmolarity changes since there was no consistent change in osmolarity with centrifugation.
5) The signal to increase ADH levels with centrifugation was not derived solely from low volume receptors in the heart, since cardiac denervation did not affect release of ADH with centrifugation.

6) The signal to increase ADH with centrifugation appeared to be under one-way control since the acceleration stimulus was sinusoidal, with a net acceleration of zero.

d. Circulating nonrepinephrine and epinephrine activity. The pressor response to acceleration in spite of a well established autonomic blockade would tend to eliminate all autonomic neural activity, including circulating catecholamines, as the source of the increase in pressure. However, the contribution of circulating catecholamines to the reflexive (unblocked) acceleration-induced pressor response remained undetermined, as did the possible influence of catecholamines on release of other pressor agents, namely renin and ADH. In addition, some recent work pointed out to us by Dr. Ziegler indicated an action of the $\alpha$-blocking agent, phenoxybenzamine, used in high dosages in our study, on pre-synaptic $\alpha$ receptors tending to greatly increase synaptic cleft concentrations of norepinephrine, perhaps to an unblockable level. Analysis of blood samples from 12 normal dogs and 6 cardiac denervated dogs (Figure B-3e) indicated:

1) Circulating levels of norepinephrine in these animals are at normal levels (487 $\pm$ 101 pg/ml), with no difference between normal and cardiac denervated dogs.
2) Acceleration did not produce a consistent change in plasma norepinephrine in either the reflexive or nonreflexive state in either normal or cardiac denervated dogs.
3) Total autonomic blockade increased circulating levels of norepinephrine by an order of magnitude in each dog (487 $\pm$ 101 pg/ml to 12, 733 $\pm$ 2489 pg/ml).

4) Circulating levels of epinephrine mimicked circulating levels of norepinephrine in response to autonomic blockade and acceleration in both normal and cardiac denervated dogs.

5) In four animals whose totally blocked pressor response was not accompanied by increased levels of ADH, it was found that in one, heart rate (and epinephrine) were elevated and in three, stroke volume (and norepinephrine) were elevated. These animals were also found to have a slight increase in stroke volume but not heart rate from the post acceleration injection of isoproterenol, indicating a diminished β blockade at the end of the run.

## DISCUSSION AND CONCLUSIONS

The pressor response to whole-body, sinusoidal acceleration seen in this study, regardless of cardiac innervation or autonomic neural effector activity appears to have both neural and hormonal components. In normally innervated dogs, prior to blockade of autonomic effectors, this increase in aortic pressure is due primarily to increased heart rate which appears to be neurally mediated since circulating levels of plasma norepinephrine and epinephrine are not significantly elevated at the time of data and blood sampling. The increase levels of ADH in this case, while significant, do not appreciably affect peripheral vascular resistance, apparently due to the buffering activity of autonomic neural effectors. In these same animals, following autonomic blockade, the acceleration-induced pressor response appears to be due to the significant increase in peripheral vascular resistance which correlates with

significantly increased levels of arginine vasopressin but not with any of the other variables measured. In this case, with autonomic buffering capability blocked, the peripheral activity of vasopressin, or some unmeasured substance, is unmasked.

The cardiac denervated dogs responded quite like the normal dogs; with the unblocked increase in aortic pressure resulting from a combined increase in heart rate and peripheral resistance, and the increase in the blocked state resulting from an increase in peripheral resistance that again correlated with increased levels of ADH, but could be due to some other, unmeasured vasoactive substance.

Few differences in control values between normal and cardiac denervated dogs in either the unblocked or autonomically blocked states were noted: cardiac denervated dogs appeared to have lower plasma volume and renin activity, elevated hematocrit and lower aortic pressure than did normal animals in the unblocked state, and in the autonomically blocked state, intrinsic heart rate was also found to be significantly lower while stroke volume was slightly, but not significantly, higher. Aside from these, other hemodynamic and neurohumoral variables appeared to be quite similar between the two groups.

There were some differences noted between male and female subjects in this study, irrespective of cardiac innervation. Four females and one male had unacceptably high levels of ADH in the control state. Nine out of ten males increased ADH in the unblocked case, while only four out of eight females did. And finally, seven out of eight females and only three of six males increased ADH in the totally blocked state, the pressor response in these four animals being due apparently to a diminished $\beta$ blockade at the end of the run which permitted an increase in stroke volume without an increase in heart rate.

When the persistence of this pressor response in the face of autonomic blockade was originally observed, we assumed the same agent would be responsible in both the unblocked and autonomically blocked cases. Therefore, our original line of pursuit was to look for increases in plasma volume with acceleration, instead we found an increase in hematocrit indicating a decreased plasma volume. We next considered plasma renin activity, which, although it changed greatly with autonomic blockade, was found not to change with acceleration in either the unblocked or autonomically blocked case. Next, we considered arginine vasopressin activity, and found there was a fairly consistent increase which was surprising since it appeared to be independent of cardiac innervation including right heart, volume sensory information. Finally, since dissection of the pressor response into its cardiac output and peripheral resistance components had indicated in the unblocked state the total heart rate dependence of the response in the normal animal and the partial heart rate dependence of the cardiac denervated animals, circulating levels of catecholamines were measured. Again, although elevated by blockade, in neither the unblocked or blocked case, were circulating levels of catecholamines consistently elevated during post acceleration data and blood sampling even though they were apparently important in the four cases in which the blockade had worn off and the pressor response was found to be due to increased stroke volume. Due to the rapid demise of catecholamines in the bloodstream, circulating levels could have been elevated during the run and assimilated by the time the blood sample was taken (1 to 3 min. after the centrifuge was shut down). However, since blood pressure and all other variables were measured at the time of blood sampling, it is this pressure for which neurohumoral correlation is sought.

Other investigators have seen similar responses to $+g_z$ stress in normal awake (8) and anesthetized (4) animals, in normal, awake man (1, 2, 10)

and in autonomically blocked animals (8) and man (2, 10). In particular, in a study which measured $+g_z$ tolerance in healthy awake men (1), many of the same variables were recorded and much the same responses (significantly increased heart rate, vasopressin and hematocrit, with nonsignificant changes in osmolarity and plasma renin activity) were seen as in the unblocked portion of the present study.

Due to the decrease in plasma volume seen by other investigators in response to $+g_z$ stress, a decrease (indicated by increased HCT) in response to $\pm g_z$ stress was not totally unexpected in the present study. However, our two measurements of plasma volume gave different results: (a) a semi-log plot of $RISA^{131}$ activity vs. time across the complete experiment for six dogs showed no consistent change in activity with either unblocked or blocked acceleration stress and (b) changes in hematocrit which have been shown to correlate inversely with plasma volume (12) indicated the decrease with acceleration stress, however, other investigators have found changes in hematocrit which did not reflect changes in plasma volume (13). Since the blood column is alternately being pooled in the lower body and then the upper body, a net efflux of plasma into the interstitial spaces is perhaps responsible for the diminished plasma volume seen by us and others (1).

Changes in plasma renin activity have been detected in response to stresses which a) tend to decrease central blood volume as with vertical tilt (increased plasma renin activity) (17), b) tend to increase central blood volume as with positive pressure breathing or water immersion (decreased plasma renin activity) (15), or c) change renal arterial pressure (18). In the latter study (18), renal arterial occlusion induced a pressor response in conscious dogs both before and after combined beta and alpha adrenergic blockade that, (in addition to increased sympathetic activity in the unblocked occlusion) was

associated with increased plasma renin activity and was blunted by the converting enzyme teprotide. Similarities between the occlusion study and the present study in control unblocked and blocked aortic pressure, heart rate, cardiac output, plasma renin activity, norepinephrine and epinephrine, and the persistence of the pressor response in the face of autonomic effector blockade, made plasma renin activity a particularly attractive candidate for the pressor agent in the present study. Since the acceleration stress used in the present study alternately pools and depletes blood in the thoracic region and also alternately raises and lowers arterial blood pressure at the renal level, any of the above mechanisms could have been invoked as responsible for an increase in plasma renin activity. However, since no increase was noted with acceleration (similar to other acceleration findings) (1), the functioning of intact baroreceptors in the present study was felt to be perhaps responsible for the lack of increase seen since it has recently been shown that functioning carotid baroreceptors can totally inhibit renin release (16). One other interesting observation was made in the present study; our cardiac denervated dogs were found to have lower control levels of plasma renin activity ($0.5 \pm 0.1$ ng/ml/hr) than did the normal dogs in our study ($2.3 \pm 0.6$ ng/ml/hr) which was not expected based on the findings of Thames et al (16) who showed that cardiopulmonary receptors with vagal afferents exert tonic inhibition of renin secretion in the presence of functioning baroreceptors. However, the present study was done on animals who had been denervated three weeks previously while the Thames study was done acutely.

We are, therefore, left with an interesting response that persisted throughout the 30 to 40 minutes of $\pm 2$ $g_z$ sinusoidal acceleration, across the

frequency range of 0.005 to 0.25 Hz (periods of 200 to 4 sec) and was independent of cardiac innervation or autonomic neural effector activity but is of greater magnitude in male subjects than in female subjects. Furthermore, at least with the substances measured here, the correlation of this response to neurohumoral vasoactive substances was best with circulatory ADH, independent of osmolarity, but was not perfect, leaving other unmeasured substances as possible candidates.

## REFERENCES (Section B)


1. Greenleaf, J. E., P. J. Brock, R. F. Haines, S. A. Rasitano, L. D. Montgomery and L. C. Keil. Effect of hypovolemia, infusion and oral rehydration on plasma electrolytes, ADH, renin activity and $+g_z$ tolerance. Aviat. Space Environ. Med., 48(8): 693-700, 1977.
2. Tyden, G. Aspects of cardiovascular reflex control in man. Acta Physiologica Scandanavica Supplementum, 448: 1-62, 1977.
3. Chimoskey, J. E. Renal hemodynamic response of unanesthetized dogs to negative acceleration. Aero. Med., 41(6): 633-637, 1970.
4. Peterson, D. F., V. S. Bishop and H. H. Erickson. Cardiovascular changes during and following 1-min. exposure to $+g_z$ stress. Aviat. Space Environ. Med., 46(6): 775-779, 1975.
5. Hershgold, E. J. and S. H. Steiner. Cardiovascular changes during acceleration stress in dogs. J. Appl. Physiol., 15(6): 1065-1068, 1960.
6. Scher, A. M. and A. C. Young. Nonlinearity in the control of blood pressure and heart rate. Ann. N. Y. Acad. Sci., 165: 722-730, 1969.
7. Stegemann, J. and V. Tibes. Sinusoidal stimulation of carotid sinus baroreceptors and peripheral blood pressure in dogs. Ann. N. Y. Acad. Sci., 165: 787-795, 1969.
8. Chimoskey, J. E. Renal hemodynamic response of unanesthetized dogs to positive acceleration. J. Appl. Physiol., 29(2): 244-248, 1970.
9. Nixon, J. V., et al. Early cardiovascular adaptation to simulated zero gravity. J. Appl. Physiol. Resp. Environ. Exercise Physiol., 46(3): 541-548, 1979.
10. Bjursted, H., G. Rosenhamer and G. Tyden. Acceleration stress and effects of propranolol on cardiovascular responses. Acta Physiol. Scand., 90: 491-500, 1974.

11. Randall, W. C., M. P. Kaye, J. X. Thomas and M. J. Barber. Intrapericardial denervation of the heart. J. Surg. Res., (1980), in press.

12. Diaz, F. J., D. R. Bransford, K. Kobayashi, S. M. Horvath and R. G. Murray. Plasma volume changes during rest and exercise in different postures in a hot, humid environment. J. Appl. Physiol: Respirat. Environ. Exercise Physiol., 47(4): 798-803, 1979.

13. Greenleaf, J. E., V. A. Convertivo and G. R. Mangseth. Plasma volume during stress in man: osmolarity and red cell volume. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 47(5): 1031-1038, 1979.

14. Yamaguchi, I. and I. J. Kopin. Plasma catecholamine and blood pressure responses to sympathetic stimulation in pitted rats.

15. Epstein, M. Cardiovascular and renal effects of head out water immersion in man.

16. Thames, M. D., M. Jarecki and D. E. Donald. Neural control of renin secretion in anesthetized dogs. Circ. Res. 42(2): 237-245, 1978.

17. Zanchetti, A. and A. Stella. Neural control of renin release. Clin. Cir. Mol. Med. 48: 215S-223S, 1975.

18. Gavras, H. and C. Liang. Acute renovascular hyptertension in conscious dogs. Circ. Res. 47: 356-365, 1980.

APPENDIX A

RESEARCH TEAM

Investigators

| | |
|---|---|
| C. F. Knapp, Ph.D. | Department of Mechanical Engineering, Wenner-Gren Biomedical Engineering Laboratory |
| D. Randall, Ph.D. | Department of Physiology and Biophysics |
| J. Evans, M.S. | Wenner-Gren Biomedical Engineering Laboratory |
| B. Kelley, Ph.D. candidate | Department of Mechanical Engineering |
| J. Charles, Ph.D. candidate | Department of Physiology and Biophysics |
| D. Bhavsar, M.S. candidate | Department of Mechanical Engineering |

Collaborators

| | |
|---|---|
| W. Griffen, M.D. | Department of Surgery, University of Kentucky Medical Center |
| T. Kotchen, M.D. | Department of Medicine, University of Kentucky Medical Center |
| G. Robertson, Ph.D. | Department of Medicine, Indiana University School of Medicine |
| M. Ziegler, Ph.D. | Departments of Pharmacological and Internal Medicine, University of Texas Medical Branch |

Technical Staff

| | |
|---|---|
| Surgical Technicians: | C. Woolfolk, D. Cloyd, D. Graham and L. Ennis |
| Instrumentation Specialists: | R. Stanifer and A. Liaupsin |
| System and Data Analysts: | E. Lowery, B.S., C. Fischer, G. Hirsch, J. Galloway and K. Fowler |

The formation of this research team is based on a general plan which integrates the advanced analytical techniques and instrumentation development capabilities of an interdisciplinary team, consisting of physiologists and

biomedical engineers, in an effort to resolve problems associated with acceleration stress. Measurements from the invasive instrumentation of the chronically implanted animal preparation of this study are essential for identifying the most meaningful variables for assessing acceleration-induced cardiovascular responses when less invasive measurements are eventually to be made on man. It is our belief that this basic research effort will provide the background for the design and implementation of human investigations, investigations which will lead to improve protective equipment and operational procedures for military personnel exposed to acceleration environments resulting from the optimal utilization of advanced aerospace systems.

## APPENDIX B

## PUBLICATIONS AND PRESENTATIONS

1. Randall, D.C., Evans, J.M., Billman, G.E., Ordway, G.A. and Knapp, C.F.: "Neural Hormonal and Intrinsic Mechanisms of Cardiac Control During Acute Coronary Occlusion in Intact Dog." J. Autonom. Nerv. Sys., in press, 1981.

2. Randall, D.C., Billman, G.E., Knapp, C.F., Evans, J.M. and Adams, J.E.: "Comparison of Heart Rate and Blood Pressure Response to Acute Coronary Occlusion in Awake vs. Sedated Dog." Submitted to J. Cardio. Pharm., 1980.

3. Knapp, C., Randall, D., and Evans, J.: "Cardiovascular Responses to ± 2G Sinusoidal Acceleration (<0.3 Hz) in Normal and Cardiac Denervated Dogs." Physiologist, 10: 70, 1979.

4. Ordway, G.A., Randall, D.C., Billman, G.E., Evans, J.M. and Knapp, C.F.: "The Effect of Exercise Conditioning Upon the Heart Rate and Blood Pressure Response to Acute Coronary Occlusion in Dog." Fed. Proc., 39: 291, 1980.

5. Randall, D.C., Evans, J.M., Billman, G.E., Ordway, G.A. and Knapp, C.F.: "Control of the Cardiac Response to Acute Coronary Occlusion in Dog." The Physiologist, 23: 28, 1980.

6. Randall, D.C., Billman, G.E., Knapp, C.F. and Evans, J.M.: "An Examination of the Possible Role of Pain and 'Anxiety' in the Canine Response to Coronary Occlusion." Pav. J. Biol. Sci., 15: 89, 1980.

7. Knapp, C.F., Randall, D.C. and Evans, J.M.: "Cardiovascular Regulation During Dynamic Acceleration Loadings in Normal and Cardiac Denervated Dogs." Proceeding of the Review of Air Force Sponsored Basic Research in Environmental and Acceleration Physiology, St. Louis University Medical Center, St. Louis, Missouri, October 2-4, 1979.

8. Knapp, C.F., Randall, D.C. and Evans, J.M.: "Cardiac Dimension Changes During Dynamic Acceleration Loadings in Normal and Cardiac Denervated Dogs." Proceeding of the Review of Air Force Sponsored Basic Research in Environmental and Acceleration Physiology, University of Kentucky, Lexington, Kentucky, September 23-25, 1980.